

Assessment of *Brassica napus* gene flow potential to wild relatives in northern South Africa

M Andriessen
22089225

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Supervisor: Prof SJ Siebert
Co-supervisor: Prof J van den Berg
Assistant Supervisor: Mr D Cilliers

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Declaration

I declare that the work presented in this *Magister Scientiae* dissertation is my own work, that it has not been submitted for any degree or examination at any other university, and that all the sources I have used or quoted have been acknowledged by complete reference.

Signature of the Student:

Mindriessen

Signature of the Supervisor:

J. J. J. J.

Signature of the Co-supervisor:

J. van den Berg

Abstract

The Brassicaceae, distributed throughout South Africa, is a family in which hybridization may occur between cultivated species and its wild and weedy relatives. Hybridization with transgenic varieties could increase the potential invasiveness and weediness of a species by conferring beneficial transgenic traits to the F_1 generation. Gene flow from transgenic *Brassica napus* to related genera is highly likely since these related genera occur frequently throughout South Africa. While no transgenic *B. napus* is approved for cultivation in South Africa, glyphosate tolerant *B. napus* is imported as food and feed. This study aimed to assess the likelihood of gene flow from *B. napus* to adjacent reproductively compatible populations with emphasis placed on their spatial distribution and overlap in populations should transgene escape occur. This study will contribute to future risk assessments of transgenic *B. napus* for cultivation in South Africa. The spatial distribution of the Brassicaceae throughout the northern provinces of South Africa was determined with the use of specimen data collected from 12 major herbaria. Seventy-six Brassicaceae species from 23 genera and 10 tribes were identified. These are widespread in the entire study area, with the most species found where (1) anthropological activity is the highest, (2) rainfall is above 300 mm, and (3) when located within the Dry Highveld Grassland and Mesic Highveld Grassland bioregions. Ten species were identified as reproductively compatible with *B. napus*. Five phytochoria were identified for the distribution of the Brassicaceae based on rainfall and mountainous environments. Four criteria were used to determine which reproductively compatible species presented the highest likelihood for gene flow to occur. Prevalence, spatial overlap, gene flow rate and distribution by anthropological activities, were identified from the literature and standardized for quarter degree grid cell data, and scored in order to identify high risk areas. The area presenting the highest risk for potential gene flow to occur was Gauteng followed by a high risk area stretching from the north-east of the study area along the eastern border through to the south-east. Possible implications of gene flow between transgenic *B. napus* and its reproductively compatible relatives were identified, and mitigation strategies suggested. Further studies is required on the potential for gene flow to occur between *B. napus* and its reproductively compatible relatives that are indigenous to South Africa.

Key words

Brassicaceae; *Brassica napus*; Canola; gene flow; GM; hybridization; risk assessment; spatial assessment

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All glory to God.

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Chapter 1: Introduction

1.1 Background

Transgene movement between related crop species (as well as introgression) is a possibility when genetically modified (GM) crops are grown in agricultural environments (Staniland *et al.* 2000; Stewart *et al.* 2003). Hybridization can only occur between a crop plant and a wild relative if a number of barriers to gene flow are crossed (McGeoch *et al.* 2009). Some barriers ensure that related taxa are not geographically proximate (McGeoch *et al.* 2009). Other barriers may include different flowering times and pollination mechanisms, reproductive incompatibility, and infertile individuals or species (McGeoch *et al.* 2009). The probability of and extent of gene flow varies in coordination with these limiting factors (Légère 2005). *Brassica napus* is a species known to overcome such barriers and hybridizes readily with wild and weedy relatives to form fertile hybrids (Légère 2005; McGeoch *et al.* 2009; Stewart *et al.* 2003).

Brassica napus has a number of characteristics that favours gene flow and the associated potential increase in weediness (Ceddia *et al.* 2009; McGeoch *et al.* 2009). These include the ability of *B. napus* to form volunteer populations with seeds that may remain in the soil seedbank for three years after harvest (D'Hertefeldt *et al.* 2008; Gulden *et al.* 2003; Smyth *et al.* 2002). Gene flow between *B. napus* and its wild and weedy relatives are affected by pollen competition, flowering synchronisation and relative density (Légère 2005). Its seeds and pollen have high mobility and it can outcross and hybridize with wild relatives, such as *B. rapa*, *Erucastrum gallicum* and *Raphanus raphanistrum* – an occurrence that has been regularly reported (Légère 2005; Warwick *et al.* 2003).

Gene flow from transgenic crops to wild relatives may have a number of negative effects. Hybridization with transgenic varieties could increase the potential invasiveness and weediness of a species (by for example conferring traits such as herbicide tolerance), thus increasing the fitness of existing invasive species (Ellstrand & Schierenbeck 2000; Halfhill *et al.* 2002; Snow *et al.* 2005; Stewart *et al.* 2003). The possible long-term ecological effects of such an invasion may be considerable. Furthermore, gene flow from transgenic plants is difficult to contain (Smyth *et al.* 2002). This has been clearly demonstrated by transgene movement in rice (high protein content, disease and insect resistance, virus resistance, herbicide resistance and salt tolerance), creeping bentgrass (glyphosate herbicide resistance), and oilseed rape (herbicide resistance) (Chen *et al.* 2004; Rieger *et al.* 2002; Warwick *et al.* 2003; Watrud *et al.* 2004; Zapiola *et al.* 2008; McGeoch *et al.* 2009).

1.2 Rationale

Brassica napus is becoming one of the most important sources of oil and protein in the world (McGeoch *et al.* 2009). It is the fourth most important oilseed crop and global production continues to increase rapidly (McGeoch *et al.* 2009). Transgenic, insect-resistant and herbicide tolerant *B. napus* varieties have been developed and tested in field experiments in North America (Légère 2005, McGeoch *et al.* 2009). Non-transgenic *B. napus* was introduced to South Africa fairly recently, with 5 000 ha planted in 1994, and 40 200 ha planted during 2005/06 (McGeoch *et al.* 2009). It has been identified as a possible crop for the production of biofuel in South Africa, and the area planted to *B. napus* may consequently increase in the future (Ceddia *et al.* 2009).

Légère (2005) defines gene flow as “the exchange of genes between different, usually related, populations through pollen transfer”. Genes and transgenes can flow between species to form hybrids if certain conditions are met (e.g. sympatry with a compatible relative) (Légère 2005). Gene flow from crops to wild relatives has led to the evolution of enhanced weediness (Ellstrand *et al.* 1999). Environmental risks posed by GM crops with herbicide tolerance, insect resistance, disease resistance and stress tolerance transgenes are of most importance (Andow & Zwahlen 2006; Snow & Palma 1997). Gene flow between related plant species, and between transgenic and non-transgenic crop varieties, may be considered a form of biological invasion (Petit 2004). GM plants have a competitive advantage over plants that do not have these traits (e.g. herbicide tolerance) (Halfhill *et al.* 2002). Gene flow associated with *B. napus* poses a potential ecological risk to the whole of South Africa. A previous study of gene flow risk in the Fynbos Biome (McGeoch *et al.* 2009) showed that further ecological risk assessment is indeed needed if transgenic *B. napus* is to be considered for release.

The Brassicaceae is present throughout South Africa, dominating in the Cape Floristic Region (Mummenhoff *et al.* 2005). The Brassicaceae is a family in which hybridization may occur readily between cultivated species (*Brassica napus* and *B. rapa*) and its wild and weedy relatives (Ellstrand *et al.* 1999). Hybridization between *B. napus* and *B. rapa* (Allainguillaume *et al.* 2006; FitzJohn *et al.* 2007; Halfhill *et al.* 2002; Hauser *et al.* 1998; Jørgensen and Andersen 1994; Warwick *et al.* 2003), and *Raphanus raphanistrum* (Gueritane *et al.* 2002; Warwick *et al.* 2003) has been reported the most frequently. Considering that the release of transgenic *B. napus* remains a possibility in southern Africa, this study aims to spatially evaluate the potential of gene flow from *Brassica* species to their wild relatives. Although this type of spatial assessment has been done for Brassicaceae crops and relatives in the Western Cape of South Africa (McGeoch *et al.* 2009; Mummenhoff *et al.* 2005), the likelihood and consequences of

gene flow in the northern provinces of South Africa have to date not been considered despite records of *B. napus* cultivation.

1.3 Objectives

1.3.1 Review scientific literature on gene flow between transgenic and non-transgenic *Brassica napus* and wild and weedy relatives, and evaluate the effects that it would have on the environment if transgenes are distributed by hybridization and introgression.

1.3.2 Quantify the diversity of wild and weedy *Brassica napus* relatives in northern South Africa and assess the spatial overlap of their distributions as a basis for understanding the potential for gene flow to occur from commercially-produced *B. napus* to its reproductively compatible relatives by:

- 1.3.2.1 Determining the spatial distribution of the Brassicaceae;
- 1.3.2.2 Providing possible environmental reasons for the distribution attained; and
- 1.3.2.3 Identifying reproductively compatible relatives and their spatial overlap.

1.3.3 Conduct a risk assessment with regards to the gene flow potential between transgenic and non-transgenic *Brassica napus* and its reproductively compatible relatives by:

- 1.3.3.1 Identifying areas with highest potential and likelihood for gene flow to occur;
- 1.3.3.2 Determining likely consequences of the exchange of genetic material between cultivated transgenic *B. napus* and its reproductively compatible relatives; and
- 1.3.3.3 Suggesting possible mitigation strategies for the prevention of undesired gene flow.

1.4 Hypotheses

1.4.1 Null hypothesis

If GM *Brassica napus* is cultivated in the northern provinces of South Africa, then there will be no gene flow potential between *B. napus* and its wild and weedy relatives.

1.4.2 Causal hypotheses

- 1.4.2.1 However, if wild and weedy relatives occur in the northern provinces of South Africa and literature confirms hybridization with *B. napus*, then there is a potential for gene flow;

1.4.2.2 But, if there is a potential for gene flow between *B. napus* and its wild and weedy relatives in the northern provinces of South Africa, then the distributions of these species have to overlap for hybridization to occur;

1.4.2.3 Therefore, if reproductively compatible Brassicaceae species occur in areas where *B. napus* is cultivated in the northern provinces of South Africa, then transgenic *B. napus* presents a risk of gene flow to wild and weedy relatives.

1.5 Format of study

Chapter 1: Introduction

Background to the study, rationale, objectives and hypotheses.

Chapter 2: Literature Study

Information regarding the research field with overviews on multiple features including the phylogeny of the Brassicaceae, characteristics and uses of *B. napus* and a short introduction to risk analysis.

Chapter 3: Method for Data Processing

Detailed methods for the study and data analyses, and original maps used for overlay.

Chapter 4: Phytogeography of the Brassicaceae

Spatial assessment of the Brassicaceae distribution patterns throughout the northern provinces of South Africa.

Chapter 5: Spatial risk assessment

Determining the likelihood of gene flow from *B. napus* to its reproductively compatible relatives throughout the northern Provinces of South Africa

Chapter 6: Conclusion and Recommendations

Concludes by providing mitigation strategies and recommendations for future studies

Appendix A: Glossary

Definitions of several terms used throughout the study.

Appendix B: Species list

All Brassicaceae species present in the study area as determined by specimen data.

Appendix C: The effect of population and surface area on specimen collection data.

Appendix D: Species occurring per QDGC in the study area

A data set indicating all QDGC's within the study area, the QDGC's used when standardizing via the Centroid Grid profile and the species present within each QDGC after standardization.

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Chapter 2: Literature Study

2.1 Origin and classification

Canola (Canadian oil, low acid) is a member of the Brassicaceae Burnett, *nom. cons.*, or Cruciferae Jussieu, *nom. cons. et nom. alt* (Al-Shehbaz *et al.* 2006; Anjum *et al.* 2012; APG III 2009). The Brassicaceae is also known as the mustard family and occurs throughout the world, except in Antarctica (Al-Shehbaz *et al.* 2006; Warwick *et al.* 2010). Their evolutionary history and tribal classification is not fully understood as recent studies have proven convergent evolution to have occurred in previous apomorphic morphological characters, disconcerting previous tribal classifications (Al-Shehbaz *et al.* 2006; Fuentes-Soriano & Al-Shehbaz 2013; Warwick *et al.* 2010). The current accepted classification includes 44 tribes (Al-Shehbaz & Warwick 2007; Fuentes-Soriano & Al-Shehbaz 2013; German & Al-Shehbaz 2008; Warwick *et al.* 2010) and 338 genera of which 8% are still unassigned to tribes, and 3709 species (Al-Shehbaz *et al.* 2006; Warwick *et al.* 2006, 2010).

Seven of the family's 338 genera are endemic to South Africa and prevail in the Cape Floristic Region: *Brachycarpaea* DC., *Chamira* Thunb., *Cycloptychis* E.Mey. ex Sond., *Heliophila* L., *Schlechteria* Bolus, *Silicularia* Compton and *Thlaspeocarpa* C.A.Sm. (Glen & Jordaan 2003; Mummenhoff *et al.* 2005). Nine tribes, 22 genera and 59 species of the Brassicaceae occur in the northern provinces of South Africa (Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North West and Northern Cape) (Winter 2006). The most genera (eight) are classified in the tribe Brassiceae DC., followed by four in Cardamineae Dumort and three in Lepideae DC. (Table 2.1) (Koch & Al-Shehbaz 2009; Warwick *et al.* 2010).

Canola originated in Canada from cultivars of *Brassica napus* L. (rapeseed or rape kale), *B. juncea* (L.) Czern. & Coss and *B. rapa* L. (Grubben & Denton 2004; Wu *et al.* 2010). For a taxon to be classified as canola the oil has to contain less than 2% erucic acid and defatted seed meals containing less than 30 micromoles per gram of aliphatic glucosinolates (Stewart *et al.* 1996; Thacker and Kirkwood 1990). *Brassica napus*, belonging to the tribe Brassiceae, is a hybrid species between *B. oleracea* L. and *B. rapa* (Anjum *et al.* 2012; Østergaard & King 2008; Warwick *et al.* 2010) and was crossed during the 1970's (Wu *et al.* 2010). *Brassica napus* is one of three allotetraploid species within the "Triangle of U": a term used to describe the formation of all possible hybridization combinations between *B. nigra* (L.) W.D.J.Koch, *B. oleracea* and *B. rapa* (Anjum *et al.* 2012; Østergaard & King 2008). During 1992, 19 varieties of *B. napus* were identified as different combinations of *B. rapa* and *B. oleracea* via chloroplast

and mitochondrial *RFLP*, forming three groups that correspond with their geographical origins (Song and Osborn 1992).

Table 2.1: Brassicaceae tribes from northern South Africa and their respective genera (Al-Shehbaz *et al.* 2006; Koch & Al-Shehbaz 2009; German & Al-Shehbaz 2008; Warwick *et al.* 2010).

Tribe	Genera
Alysseae DC.	<i>Lobularia</i> Desv.
Anchonieae DC.	<i>Matthiola</i> R.Br.
Brassicaceae DC.	<i>Brassica</i> L., <i>Crambe</i> L., <i>Diplotaxis</i> DC., <i>Eruca</i> Mill., <i>Erucastrum</i> (DC.) C.Presl, <i>Raphanus</i> L., <i>Rapistrum</i> Crantz, <i>Sinapis</i> L.
Camelineae DC.	<i>Camelina</i> Crantz, <i>Capsella</i> Medik.
Cardamineae Dumort.	<i>Aplanodes</i> Marais, <i>Cardamine</i> L., <i>Nasturtium</i> R.Br., <i>Rorippa</i> Scop.
Descurainieae Al-Shehbaz, Beilstein & E.A. Kellogg	<i>Descurainia</i> Webb & Berthel.
Heliophileae DC.	<i>Heliophila</i> L.
Lepidieae DC.	<i>Cardaria</i> Desv., <i>Coronopus</i> Zinn, <i>Lepidium</i> L.
Sisymbrieae DC.	<i>Sisymbrium</i> L.

2.2 Characteristics

Brassica napus grows up to 1.5 m high (Grubben & Denton 2004), although different crop varieties may differ in height. *Brassica napus* differs from its closest relative, *B. rapa*, by its bluish green leaves, where the latter has bright green leaves, and its buds overtop the open flowers in the inflorescence that can be up to 600 mm in length (Grubben & Denton 2004). The flowers have a cross shape (Figure 2.1) that is formed by four diagonally opposite petals of the corolla (Anjum *et al.* 2012; Franzke *et al.* 2011). From there the alternative name, Cruciferae, for the Brassicaceae which is derived from the word “crucifer” (Anjum *et al.* 2012; Franzke *et al.* 2011). The dominant allele petal colour of *B. napus* is yellow, resulting in bright to dark yellow, bisexual flowers that are 10–15 mm in length (Grubben & Denton 2004; Séguin-Swartz *et al.* 1997). The fruits are 45–110 x 3–4 mm and contain up to 30 blue-black to dark brown seeds, 1.5–2.5 mm in diameter (Grubben & Denton 2004).



Figure 2.1: *Brassica napus* flower (left), and inflorescence and seed pods (right). (Photos by B. Greyvenstein.)

2.3 Uses

The Brassicaceae comprises of species that are commercially, economically and scientifically important (Anjum *et al.* 2012; Koch & Al-Shehbaz 2009). Five thousand hectares of *B. napus* was planted in the year that the crop was introduced to South Africa during 1994, and 25 000 ha during 1999 (Mosiane *et al.* 2003). Most farmers in southern Africa (Figure 2.2) use seeds of *B. napus* that have been passed down for generations, but many farmers are replacing their stock with higher yielding *B. napus* hybrids (Grubben & Denton 2004). *Brassica napus* grows best in cool temperatures and is best adapted to grow in the highlands during the colder seasons in southern Africa (Grubben & Denton 2004).

Brassica napus seed contain a high concentration of micronutrients (Grubben & Denton 2004). It contains low levels of erucic acid – glucosinolates that form antioxidant and anticancer compounds when in its prepared form – and iron that is easily digested (Grubben & Denton 2004; Wu *et al.* 2010).

The leaves of *B. napus* are consumed as a vegetable, often accompanying starch or salad (Grubben & Denton 2004). It is commercially planted as a crop to produce high quality edible oil which may additionally be used as lamp oil or to make soap (Grubben & Denton 2004; Hardy 2004; Wu *et al.* 2010). Meal derived of *B. napus* is often used as feed for birds and the high quality components are used as replacement for imported fish meal and oil cake for livestock feed (Grubben & Denton 2004; Hardy 2004; Wu *et al.* 2010).

Brassica napus is often planted as a rotation crop in the Western Cape of South Africa (Tewoldemedhin *et al.* 2006). This serves to protect cereal grain crops such as wheat (*Triticum aestivum* L.) against soil-borne diseases, such as crown rot caused by *Fusarium pseudograminearum* Aoki & O'Donnell, and weeds (Hardy 2004; Lamprecht *et al.* 2006; Tewoldemedhin *et al.* 2006).



Figure 2.2: *Brassica napus* fields in the Western Cape Province, South Africa. (Photo by B. Greyvenstein.)

2.4 Genetically modified crops

Genetically modified (GM) crops are modified to possess specific characteristics and/or desired traits to improve yield and nutrition (Barton & Dracup 2000). This is done by genetically engineering (moving and modifying) genes to achieve an ultimately better and improved plant product (Barton & Dracup 2000). A transgenic organism contains genes that have been transferred from one organism to another and these genes function in the same way and yield the same results (Nabors 2004). Herbicide tolerance and insect resistance are some of the well-known traits of GM crops.

2.4.1 Herbicide resistance/tolerance

Herbicide resistance is a trait that allows crops to gain an advantage over weeds that compete for the same resources (Ellstrand & Schierenbeck 2000). This trait brings considerable economic value to the agribusiness industry (Snow & Palma 1997). Herbicide resistance allows specific herbicides to be used on a crop field, killing all plants that are not resistant (Nabors 2004). Herbicide resistant crops may be genetically engineered to contain a bacterial gene that does not respond to glyphosate, the main component of Roundup® which inhibits the syntheses of phenylalanine and tryptophan (Nabors 2004; Stargrove & Stargrove 2008).

2.4.2 Pest and disease resistance

Insect resistant crops contain genes from *Bacillus thuringiensis* Berliner, a bacterium that contains genes that act as natural insecticides/pesticides (Barton & Dracup 2000). The resulting insect resistant (*Bt*) crops, such as varieties of potatoes, tomatoes and rice, are resistant to insect pests (Nabors 2004). Pests may be paralyzed or killed when feeding from an insect resistant crop (Snow & Palma 1997). Viral resistance requires different transgenes for the different varieties of viruses, whereas a single transgene may also be effective against multiple bacterial and fungal diseases (Snow & Palma 1997).

2.5 Transgenic crops in South Africa

The Genetically Modified Organisms Act of South Africa (1997) sets standards and rules for the movement and use of genetically modified organisms to ensure the sustainability of biodiversity (South Africa 1997; Gruère & Sengupta 2008). Regulations within the Act include the prevention of possible human induced disasters, the control of activities that involve GM organisms, and to enforce requirements and criteria for risk assessments.

South Africa produces insect resistant and herbicide tolerant cotton (*Gossypium hirsutum* L.), insect resistant and herbicide tolerant maize (*Zea mays* L.), and herbicide tolerant soybeans (*Glycine max* (L.) Merr.) (Gouse 2010; Gruère & Sengupta 2008; Karembu *et al.* 2009; Novy *et al.* 2011). Cotton was the first GM crop to be planted in South Africa during 1997/98, making South Africa the first African country to commercialize GM crops (Gouse 2013; Karembu *et al.* 2009). In 2008, 92% of cotton in South Africa was GM, 83% of which were both herbicide tolerant and insect resistant (Karembu *et al.* 2009). As maize is the most important field crop in South Africa (Gouse 2013), the first *Bt* yellow maize was commercialized during 1998 and white maize during 2001, herbicide tolerant maize during 2002, and maize with both *Bt* and herbicide

tolerance during 2007 (Gouse 2013). Herbicide tolerant soybeans were commercialized during 2002 (Gruère & Sengupta 2008).

The most important (major) crops in Africa are root crops (e.g. potatoes), cereals (e.g. maize and wheat), fruit (e.g. grapes, mangoes and oranges), vegetables (e.g. cabbage), oilseeds (e.g. sunflower), sugar cane, pulses, cocoa beans, cotton lint, coffee, nuts and tea (Abate *et al.* 2000). On-going GM research in South Africa during 2009 included drought or herbicide tolerant and/or insect resistant maize, starch enhanced cassava (*Manihot esculenta* Crantz), insect resistant and/or herbicide tolerant cotton, insect resistant potato (*Solanum tuberosum* L.), and sugarcane (*Saccharum officinarum* L.) containing transgenes encoding for alternative sweetener (isomaltulose) (ISAAA 2015; Karembu *et al.* 2009).

2.6 Genetically modified *Brassica napus*

Brassica napus was selectively propagated to contain advantageous traits long before it was genetically modified (Grubben & Denton 2004; Senior & Dale 2002). Japanese seed companies have incorporated the heat tolerance of *B. oleracea*, the finer taste of *B. rapa*, and the disease resistance from both into the first generation (F₁) hybrid *B. napus* population (Grubben & Denton 2004). Diseases that *B. napus* hybrids may be resistant to include alternaria leaf spot (*Alternaria brassicicola*), black rot (*Xanthomonas campestris* pv. *campestris*), blackleg (*Leptosphaeria maculans*) (albeit recessive), clubroot (*Plasmodiophora brassicae*), downy mildew (*Peronospora parasitica*), turnip mosaic virus and white rust (*Albugo candida*) (Séguin-Swartz *et al.* 1997), all of which are of concern in South Africa (Alvarez 2000; Dixon 2009; Fitt *et al.* 2006; Morris and Knox-Davies 1980; Nyvall 1989; Peever *et al.* 2004). Pests that *B. napus* hybrids may be resistant to include the cabbage aphid (*Brevicoryne brassicae*) and the flea beetle (*Phyllotreta cruciferae*) (Séguin-Swartz *et al.* 1997).

Genetically modified *B. napus* that has been encoded for herbicide resistance, insect or fungal resistance (in 1994 in Copenhagen, Denmark (Jørgensen and Andersen 1994), and other traits such as cold and stress tolerance, have been evaluated in field tests (Snow & Palma 1997). The first transgenic *B. napus* was commercially released in Canada during 1995, followed by the United States of America during 1998 (Londo *et al.* 2014; Schafer *et al.* 2011). Its varieties were encoded for glyphosate (Roundup Ready®) and/or glyphosate-ammonium (Liberty Link®) herbicide resistance (Londo *et al.* 2014; Schafer *et al.* 2011). More than 90% of *B. napus* cultivated in the United States were genetically modified during 2011 (Schafer *et al.* 2011) and 98% in Canada during 2006 (Knispel *et al.* 2008). Transgenic *B. napus* is preferred above non-transgenic varieties because of the increase and ease of production (Knispel *et al.* 2008). Types

of transgenic *B. napus* that have been approved for food and/or feed or cultivation across the world are indicated in Table 2.2 (ISAAA 2014).

Imports of genetically modified *B. napus* (as food and feed) to South Africa was approved during 2001 (Gruère & Sengupta 2008:6; ISAAA 2014) (Table 2.2). Between 2001 and 2006, only 1% of imported *Brassica napus* oil was GM (by Bayer CropScience), and 0% of *Brassica napus* products (excluding oil) (Gruère & Sengupta 2008). These imports coincide with four GM-events, of which one of the varieties encode for herbicide tolerance and the remaining three for stacked traits of herbicide tolerance and a pollination control system (ISAAA 2014) (Table 2.2). Monsanto, an agrochemical company, applied for a field trial release of RT73 *Brassica napus* that encoded for herbicide resistance (Roundup™) to South Africa during 2009 (Stafford 2009), but Monsanto withdrew their application during 2010 (ACB 2010).

2.7 Gene flow

Gene flow is defined as a transfer of genetic material that occurs when alleles, gametes, or individuals are exchanged or moved from one population to another, either by natural or anthropogenic means (Clement *et al.* 2009:118; Légère 2005; Nabors 2004; Slatkin 1987). Barriers and reproductive isolation mechanisms prevent species from exchanging genetic material (Slatkin 1987). Gene flow occurs when certain environmental requirements are met to overcome reproductive isolation mechanisms, e.g., species are morphologically similar, occur in the same geographical location, are adapted to similar environmental factors (variations in rainfall, soil conditions and temperatures), and have coinciding flowering periods (Nabors 2004; Rieger *et al.* 2002). Gene flow may result in the formation of a new species over time or prevent species from changing by maintaining the gene variation by countering natural selection and genetic drift (Cresswell *et al.* 2002; Slatkin 1987).

Gene flow does not occur between different populations and the gene pool is retained when species are reproductively isolated (Slatkin 1987). When multiple populations share the same environmental requirements and barriers are overcome, then hybridization may occur (Levin *et al.* 1996). Hybridization (cross-breeding) occurs when genetic material is exchanged between two different, previously reproductively isolated, populations (Clement *et al.* 2009; Levin *et al.* 1996). Gene flow may occur via pollen transfer or seed dispersal (Gulden *et al.* 2003; Levin & Kerster 1974). Within this study, hybridization refers to all exchange of genetic material between different species and different populations and variations within the same species.

2.8 Resistant weeds

Invasiveness is the distribution and persistence of a group of individuals in a new environment (Ellstrand & Schierenbeck 2000). *Brassica napus* is an invasive cultivated species in temperate regions throughout the world (Pessel *et al.* 2001). Many of its family members are wild and weedy (Gueritaine *et al.* 2002) and present in or around its cultivated areas (Warwick *et al.* 2003). Weediness allows a plant to thrive in a disturbed environment by containing traits such as swift early-seasonal growth, producing more seeds, having more means to distribute seeds, seed dormancy, and/or forming volunteers in the seed bank, whilst in opposition with cultivated plants (Kwit *et al.* 2011).

Non-transgenic *B. napus* has many characteristics that favour gene flow (Gueritaine *et al.* 2002; Gulden *et al.* 2003; Légère 2005; Warwick *et al.* 2003). *Brassica napus* produces large amounts of pollen (Gueritaine *et al.* 2002), is 20–40% allogamous (degree of outcrossing) (Gueritaine *et al.* 2002; Warwick *et al.* 2003) and has been proven to outcross with species it is closely related to (Gueritaine *et al.* 2002; Warwick *et al.* 2003). *Brassica napus* has potential secondary seed dormancy that allows its seeds to persist in the seedbank for up to ten years and forming volunteer populations (D’Hertefeldt *et al.* 2008; Gulden *et al.* 2003; Warwick *et al.* 2003). When seeds germinate after long periods of time in the seedbank, the plants are called “volunteers” (Reagon & Snow 2006).

Genes may gradually be transferred by pollen flow from herbicide resistant transgene crop varieties to its wild relatives that grow near the cultivated fields (Nabors 2004; Smyth *et al.* 2002). Wild and weedy relatives may gain the same traits that the transgenic ascendants had, such as herbicide tolerance, when hybridization occurs (Halfhill *et al.* 2002; Légère 2005; Nabors 2004; Smyth *et al.* 2002). Traits of *B. napus* in combination with transgenes and traits of its family members may have significant consequences (Stewart *et al.* 1997), such as an increase in difficulty for removal of these species from unwanted locations (Légère 2005) and an increase in the potential for gene flow to occur (Pessel *et al.* 2001). Gene flow may occur within escaped *B. napus* populations and allow these populations to obtain multiple transgenic traits (Knispel *et al.* 2008), and form highly competitive herbicide resistant weeds (Nabors 2004; Smyth *et al.* 2002). Beckie *et al.* (2003) and Hall *et al.* (2000) further confirmed this view by stating that transgenes from different *B. napus* varieties frequently stack in volunteer populations in western Canada at distances further than 50 m from the source field, allowing these stacked-trait volunteers to prevail in large areas.

In western Canada, Knispel *et al.* (2008) found that transgenic *B. napus* was not confined to the agricultural fields that it was planted in. Escaped transgenic *B. napus* containing multiple-

herbicide resistant seeds were found along roadsides in Canada (Knispel *et al.* 2008) and Japan (Aono *et al.* 2006). Furthermore, with the ability of these seeds to induce secondary dormancy (Gulden *et al.* 2003; Pessel *et al.* 2001; Warwick *et al.* 2003) and populations to persist for four (Gulden *et al.* 2003; Hall *et al.* 2000), eight to nine (Pessel *et al.* 2001), and up to ten years without intervention (D'Hertefeldt *et al.* 2008; Pessel *et al.* 2001) the risk of gene flow to occur between *B. napus* and its wild and weedy relatives is further increased.

Genetically modified crop plants may become weeds themselves in their areas of cultivation due to the respective transgenes providing them with a competitive advantage, and thus an increase in species fitness, over other crops (Ellstrand & Schierenbeck 2000; Nabors 2004; Stewart *et al.* 1996). For this to occur, the species has to become naturalized or be able to hybridize with other related individuals within the same area, and selection pressure should be present (Stewart *et al.* 1996).

Transgene introgression occur when genes from one modified population is permanently fixed into the genetic background of another population by means of hybridization and backcrossing over several generations (Kwit *et al.* 2011; Stewart *et al.* 2003). Backcrossing is necessary for introgression to occur (FitzJohn *et al.* 2007). Introgression differs from hybridization in that the former is a permanent effect that occurs over a long period of time, whilst hybridization and gene flow occurs in every generation and is not necessarily a permanent change (Stewart *et al.* 2003). Hybridization, and possibly transgene introgression, may occur between *B. napus* and *B. rapa* (Allainguillaume *et al.* 2006; FitzJohn *et al.* 2007; Halfhill *et al.* 2002; Hauser *et al.* 1998; Jørgensen and Andersen 1994; Warwick *et al.* 2003), *Erucastrum gallicum* (Willd.) O.E. Schulz (Warwick *et al.* 2003), *Raphanus raphanistrum* L. (Gueritane *et al.* 2002; Warwick *et al.* 2003), and *Sinapis arvensis* L. (Warwick *et al.* 2003) and several other species within the Brassicaceae (FitzJohn *et al.* 2007).

The addition of transgenes to a population may or may not increase the fitness of the descendent generation (Ellstrand & Schierenbeck 2000; Halfhill *et al.* 2002). Even so, the exchange of genes between genetically modified species and their wild and weedy relatives may pose a risk to other species in the environment due to the addition of novelty genes (Ellstrand & Schierenbeck 2000; Stewart *et al.* 1997).

2.9 Risk analysis

A risk analysis is composed of risk assessment, risk management and risk communication (Johnson *et al.* 2006). The risk assessment process involves the identification and description of sources that may pose a risk to humans and the environment, the effects and likelihood thereof, as well as the potential negative consequences that gene flow presents (Andow & Hilbeck 2004;

Johnson *et al.* 2006; McGeoch *et al.* 2009). A risk assessment crosses the boundaries between conservation ecology and invasion biology (Petit 2004) by integrating both to accomplish a single goal.

In this study, a spatial risk assessment is conducted with the use of spatial data obtained from the spatial assessment (Chapter 4: Phytogeography of the Brassicaceae). The distribution of species that are known to be reproductively compatible with *B. napus* is identified as a means to determine the prevalence and spatial overlap that these relatives may have. This data, along with each species' likelihood for gene flow to occur and potential for distribution by anthropological activity is used as a means to determine areas where the likelihood for potential gene flow is highest (Chapter 5: Spatial Risk Assessment). Possible effects of gene flow between transgenic *B. napus* and its wild and weedy relatives are identified and potential mitigation strategies presented (Chapter 6: Conclusion and Recommendations) as part of the spatial risk assessment. The information obtained through this study will contribute to future decisions regarding the cultivation of transgenic *B. napus* in the northern provinces of South Africa.

Table 2.2: Transgenic *Brassica napus* events that have been approved for commercial use (food and/or feed) or cultivation across the world (ISAAA 2014).

Trade name	Developer	Transgene	Transgene source	Transgene trait	Country and year approved		
					Country	Year	Type
InVigor™ Canola	Bayer CropScience	pat (syn)	<i>Streptomyces viridochromogenes</i>	Glufosinate herbicide tolerance	¹ Canada	1996	Feed and Cultivation
					¹ Canada	1997	Food
					¹ USA	1998	All
					¹ Japan	2001	Food
					¹ Mexico	2001	Food
					¹ Australia	2002	Food
					¹ China	2002	Food and Feed
					¹ New Zealand	2002	Food
					¹ Australia	2003	Cultivation
					¹ Japan	2003	Feed
					¹ South Korea	2005	Food and Feed
					¹ Japan	2007	Cultivation
InVigor™ Canola	Bayer CropScience	bar ¹ ; barnase ² ;	<i>Streptomyces hygroscopicus</i> ¹ ; <i>Bacillus amyloliquefaciens</i> ² ;	Glufosinate herbicide tolerance ¹ ; Male sterility ² ;	² Canada	1996	Feed and Cultivation
					² Canada	1997	Food
					² USA	1998	Food and Feed
					² USA	1999	Cultivation
					² Japan	2001	Food
					² Australia	2002	Food
					² New Zealand	2002	Food
					² Australia	2003	Cultivation
					² Japan	2003	Feed
					² Japan	2006	Cultivation
					² European Union	2007	Feed
					² South Korea	2012	Feed
InVigor™ Canola	Bayer CropScience	bar ¹ ; barstar ²	<i>Streptomyces hygroscopicus</i> ¹ ; <i>Bacillus amyloliquefaciens</i> ² ;	Glufosinate herbicide tolerance ¹ ; Fertility restoration ²	³ Canada	1996	Feed and Cultivation
					³ Canada	1997	Food
					³ USA	1998	Food and Feed
					³ USA	1999	Cultivation
					³ Japan	2001	Food
					³ Australia	2002	Food
					¹³ New Zealand	2002	Food
					³ Australia	2003	Cultivation

					³ Japan	2003	Feed
					³ European Union	2007	Feed
					³ Japan	2007	Cultivation
					³ Mexico	2007	Food
					³ South Korea	2012	Feed
					³ European Union	2013	Food
					³ South Korea	2013	Food
InVigor™ Canola	Bayer CropScience	bar ¹ ; barnase ² ; barstar ³ ;	<i>Streptomyces hygrosopicus</i> ¹ ; <i>Bacillus amyloliquefaciens</i> ² ; <i>Bacillus amyloliquefaciens</i> ³ ;	Glufosinate herbicide tolerance ¹ ; Male sterility ² ; Fertility restoration ³ ;	⁴ Canada	1996	Feed and Cultivation
					⁴ Canada	1997	Food
					⁴ Japan	2001	Food
					⁴ South Africa	2001	Food and Feed
					⁴ Australia	2002	Food
					⁴ China	2002	Food and Feed
					⁴ New Zealand	2002	Food
					⁴ Australia	2003	Cultivation
					⁴ Japan	2003	Feed
					⁴ Mexico	2004	Food
					⁴ South Korea	2005	Food and Feed
					⁴ European Union	2007	Feed
					⁴ Japan	2007	Cultivation
InVigor™ Canola	Bayer CropScience	bar ¹ ; barstar ² ; nptII ³	<i>Streptomyces hygrosopicus</i> ¹ ; <i>Bacillus amyloliquefaciens</i> ² ; <i>Escherichia coli</i> ³	Glufosinate herbicide tolerance ¹ ; Fertility restoration ² ; Antibiotic resistance ³	⁵ Canada	1994	Food
					⁵ Canada	1995	Feed and Cultivation
					⁶ Canada	1995	All
					⁵ European Union	1996	Feed
					^{5,6} USA	1996	Food and Feed
					⁵ European Union	1997	Food
					⁶ European Union	1997	Food and Feed
					⁵ Mexico	1999	Food
					^{5,6} Australia	2002	Food
					^{5,6} China	2002	Food and Feed
					^{5,6} New Zealand	2002	Food
					^{5,6} USA	2002	Cultivation
					⁵ Australia	2003	Cultivation
InVigor™ Canola	Bayer CropScience	bar ¹ ; barnase ² ; nptII ³	<i>Streptomyces hygrosopicus</i> ¹ ; <i>Bacillus amyloliquefaciens</i> ² ; <i>Escherichia coli</i> ³	Glufosinate herbicide tolerance ¹ ; Male sterility ² ; Antibiotic resistance ³	⁷ Canada	1995	All
					⁷ European Union	1996	Feed
					⁷ USA	1996	Food and Feed
					⁷ European Union	1997	Food
					⁷ Mexico	1999	Food
					⁷ Australia	2002	Food
					⁷ China	2002	Food and Feed

					⁷ New Zealand	2002	Food
					⁷ USA	2002	Cultivation
					⁷ Australia	2003	Cultivation
InVigor™ Canola	Bayer CropScience	bar ¹ ; barnase ² ; barstar ³ ; nptII ⁴	<i>Streptomyces hygroscopicus</i> ¹ ; <i>Bacillus amyloliquefaciens</i> ² ; <i>Bacillus amyloliquefaciens</i> ³ ; <i>Escherichia coli</i> ⁴	Glufosinate herbicide tolerance ¹ ; Male sterility ² ; Fertility restoration ³ ; Antibiotic resistance ⁴	¹⁰ Canada	1994	Food
					¹⁰ Canada	1995	Feed and Cultivation
					⁹ Canada	1995	All
					¹⁰ European Union	1996	Feed
					¹⁰ European Union	1997	Food
					⁹ European Union	1997	Food and Feed
					¹⁰ Mexico	1999	Food
					^{9,10} Japan	2001	Food
					^{9,10} South Africa	2001	Food and Feed
					^{9,10} Australia	2002	Food
					^{9,10} China	2002	Food and Feed
					^{9,10} New Zealand	2002	Food
					^{9,10} Australia	2003	Cultivation
					^{9,10} Japan	2003	Feed
					^{9,10} South Korea	2005	Food
Laurical™ Canola	Monsanto Company	te ¹ ; nptII ²	<i>Umbellularia californica</i> ¹ ; <i>Escherichia coli</i> ²	Modified oil / Fatty acid ¹ ; Antibiotic resistance ²	¹² USA	1994	Feed and Cultivation
					¹¹ USA	1994	All
					^{11,12} Canada	1996	All
Liberty Link™ Independence™	Bayer CropScience	bar ¹ ; nptII ²	<i>Streptomyces hygroscopicus</i> ¹ ; <i>Escherichia coli</i> ²	Glufosinate herbicide tolerance ¹ ; Antibiotic resistance ²	¹³ USA	1995	All
					¹³ Japan	2001	Food
					¹³ China	2002	Food and Feed
					¹³ Japan	2003	Feed
Liberty Link™ Innovator™	Bayer CropScience	bar ¹ ; nptII ²	<i>Streptomyces hygroscopicus</i> ¹ ; <i>Escherichia coli</i> ²	Glufosinate herbicide tolerance ¹ ; Antibiotic resistance ²	¹³ Japan	2007	Cultivation
					¹⁴ Canada	1995	All
					¹⁴ USA	1995	Food and Feed
					¹⁴ European Union	1997	Food
					¹⁴ European Union	1998	Feed
					¹⁴ Mexico	1999	Food
					¹⁴ Japan	2001	Food
					¹⁴ South Africa	2001	Food and Feed
					¹⁴ Australia	2002	Food
					¹⁴ China	2002	Food and Feed
					¹⁴ New Zealand	2002	Food
					¹⁴ USA	2002	Cultivation
					¹⁴ Australia	2003	Cultivation

					¹⁴ Japan	2003	Feed
					¹⁴ South Korea	2005	Food
					¹⁴ Japan	2007	Cultivation
					¹⁴ South Korea	2008	Feed
Navigator™ Canola	Bayer CropScience	bxn	<i>Klebsiella pneumoniae</i> subsp. <i>ozaenae</i>	Oxynil herbicide tolerance	¹⁵ Canada	1997	All
					¹⁵ USA	1999	Food
					¹⁵ Japan	2001	Food
					¹⁵ Australia	2002	Food
					¹⁵ China	2002	Food and Feed
					¹⁵ New Zealand	2002	Food
					¹⁵ Japan	2003	Feed
					¹⁵ Japan	2008	Cultivation
Not Available	DuPont	gat4621	<i>Bacillus licheniformis</i>	Glyphosate herbicide tolerance	¹⁶ Canada	2012	All
Not Available	Bayer CropScience	bar ¹ ; barnase ² ; barstar ³ ;	<i>Streptomyces hygroscopicus</i> ¹ ; <i>Bacillus amyloliquefaciens</i> ² ; <i>Bacillus amyloliquefaciens</i> ³ ;	Glufosinate herbicide tolerance ¹ ; Male sterility ² ; Fertility restoration ³ ;	^{17,18,19,20} Japan	2001	Food
					^{17,18,19,20} Japan	2003	Feed
Not Available	Bayer CropScience	bar ¹ ; barnase ² ; barstar ³ ; cp4 epsps (aroA:CP4) ⁴ ; goxv247 ⁵	<i>Streptomyces hygroscopicus</i> ¹ ; <i>Bacillus amyloliquefaciens</i> ² ; <i>Bacillus amyloliquefaciens</i> ³ ; <i>Agrobacterium tumefaciens</i> ⁴ ; <i>Ochrobactrum anthropi</i> ⁵	Glufosinate herbicide tolerance ¹ ; Male sterility ² ; Fertility restoration ³ ; Glyphosate herbicide tolerance ^{4,5}	²¹ Japan	2010	Food
					²¹ Japan	2011	Feed
					²¹ Mexico	2011	Food
					²¹ Japan	2012	Cultivation
Optimum® Gly Canola	DuPont	gat4621	<i>Bacillus licheniformis</i>	Glyphosate herbicide tolerance	²² Canada	2012	All
					²² Mexico	2012	Food
					²² USA	2012	Food and Feed
					²² USA	2013	Cultivation
					²² Australia	2014	Food
					²² New Zealand	2014	Food
Phytaseed™ Canola	BASF	nptII ¹ ; phyA ²	<i>Escherichia coli</i> ¹ ; <i>Aspergillus niger</i> ²	Antibiotic resistance ¹ ; Phytase production ²	²³ USA	1998	Cultivation
					^{23,24,25,26,27} USA	1999	Food and Feed
Roundup Ready™ Canola	Monsanto Company	cp4 epsps (aroA:CP4) ¹ ; goxv247 ²	<i>Agrobacterium tumefaciens</i> ¹ ; <i>Ochrobactrum anthropi</i> ²	Glyphosate herbicide tolerance	²⁹ Canada	1994	Food
					²⁹ Canada	1995	Feed
					²⁹ USA	1995	Food and Feed
					²⁹ Mexico	1996	Food
					²⁸ Canada	1997	All
					²⁹ European Union	1997	Food

					²⁹ USA	1999	Cultivation
					²⁹ Australia	2000	Food
					²⁹ New Zealand	2000	Food
					^{28,29} Japan	2001	Food
					²⁹ China	2002	Food and Feed
					²⁸ USA	2002	Food and Feed
					²⁹ Australia	2003	Cultivation
					^{28,29} Japan	2003	Feed
					²⁹ Philippines	2003	Food and Feed
					²⁹ South Korea	2003	Food
					²⁸ USA	2003	Cultivation
					²⁹ South Korea	2005	Feed
					^{28,29} Japan	2006	Cultivation
					²⁸ Chile	2007	Cultivation
					²⁹ European Union	2007	Feed
					²⁹ Singapore	2014	Food
TruFlex™ Roundup Ready™ Canola	Monsanto Company	cp4 epsps (aroA:CP 4)	<i>Agrobacterium tumefaciens</i>	Glyphosate herbicide tolerance	³⁰ Australia	2012	Food and Feed
					³⁰ Canada	2012	All
					³⁰ New Zealand	2012	Food and Feed
					³⁰ USA	2012	Food and Feed
					³⁰ Japan	2013	All
					³⁰ Mexico	2013	Food
					³⁰ USA	2013	Cultivation
					³⁰ South Korea	2014	Feed

Notes: [Developers] Bayer CropScience (including fully and partly owned companies), DuPont (Pioneer Hi-Bred International Inc.), Monsanto Company (including fully and partly owned companies); [Event name] ¹ HCN28 (T45), ² MS8, ³ RF3, ⁴ MS8 x RF3, ⁵ RF1 (B93-101), ⁶ RF2 (B94-2), ⁷ MS1 (B91-4), ⁸ MS1 x RF 3, ⁹ MS1 x RF2 (PGS2), ¹⁰ MS1 x RF1 (PGS1), ¹¹ 23-18-17 (Event 18), ¹² 23-198 (Event 23), ¹³ HCN10 (Topas 19/2), ¹⁴ HCN92 (Topas 19/2), ¹⁵ OXY-235, ¹⁶ 61061, ¹⁷ PHY14, ¹⁸ PHY23, ¹⁹ PHY35, ²⁰ PHY36, ²¹ MS8 x RF3 x GT73 (RT73), ²² 73496, ²³ MPS963, ²⁴ MPS961, ²⁵ MPS962, ²⁶ MPS964, ²⁷ MPS965, ²⁸ GT200 (RT200), ²⁹ GT73 (RT73), ³⁰ MON88302.

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Chapter 3: Method for Data Processing

3.1 Introduction

A spatial assessment of the Brassicaceae and a qualitative spatial risk assessment with regards to gene flow potential between *B. napus* and its wild and weedy relatives was conducted in the northern provinces of South Africa; specifically Gauteng, Limpopo, Mpumalanga, North-West, and everything north of 29° S in the Free State and Northern Cape provinces (Figure 3.1).

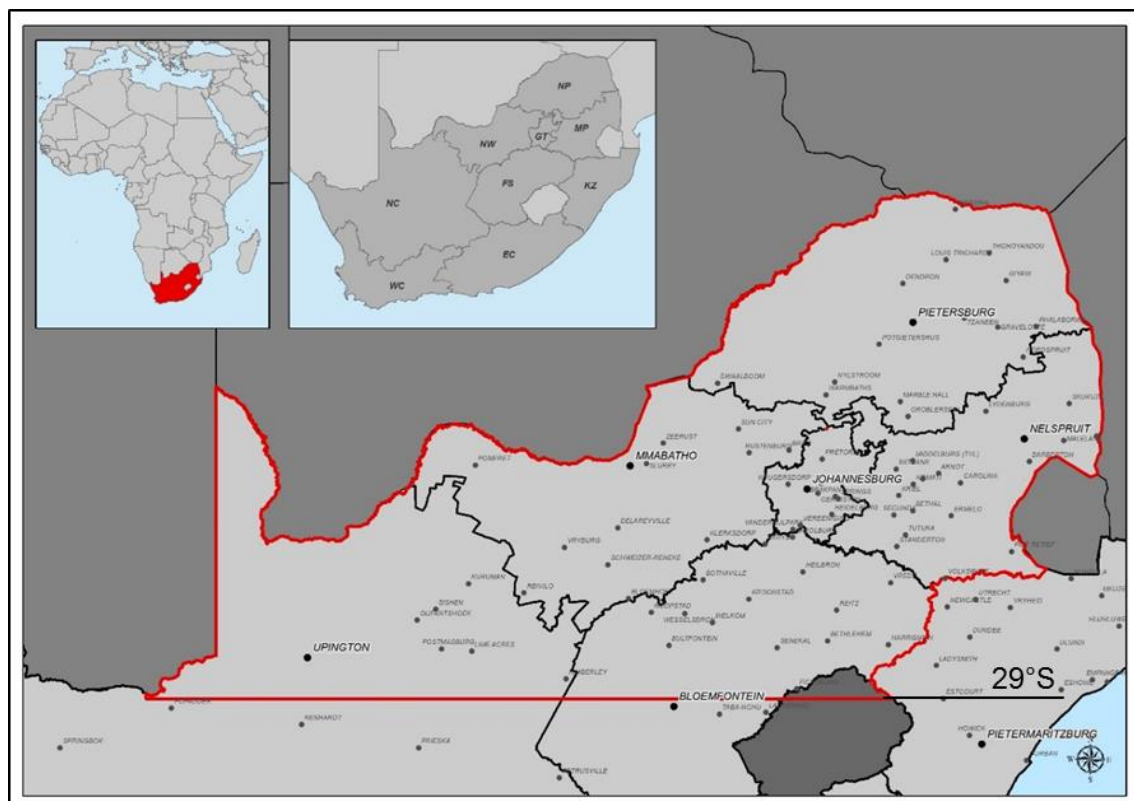


Figure 3.1: Study area in the northern provinces of South Africa indicated with a red border.

3.2 Defining the borders of the study area

The study area was divided into quarter-degree grid cells (QDGC). Grid cells overlapping two or more provinces were assigned to a specific province, depending upon which side the bulk of the surface was present in. This was determined with Google Earth (Figure 3.2a), polygons (Figure 3.2b), and Google Earth tool: Earth Point for calculations (Figure 3.2c, d, e) for each QDGC in the study area (Figure 3. 2f, g). QDGC's that fell outside the study area were removed.

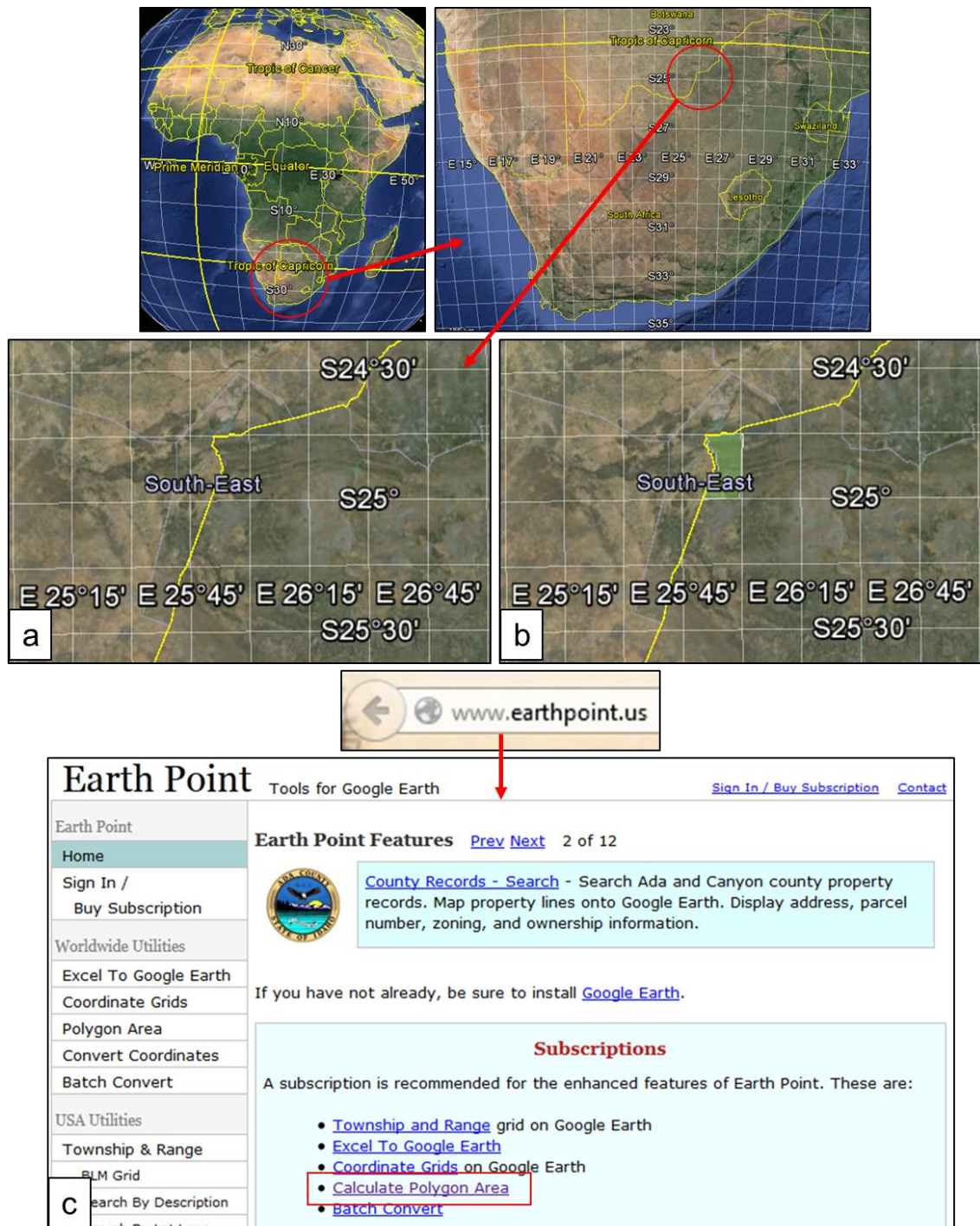


Figure 3.2: Determining grid surface area for inclusion of QDGC in the study area and allocation to provinces. QDGC as seen in Google Earth (a), and using polygons to select a single grid surface area (b). The selected surface area was calculated using Google Earth tool: Earth Point (c).

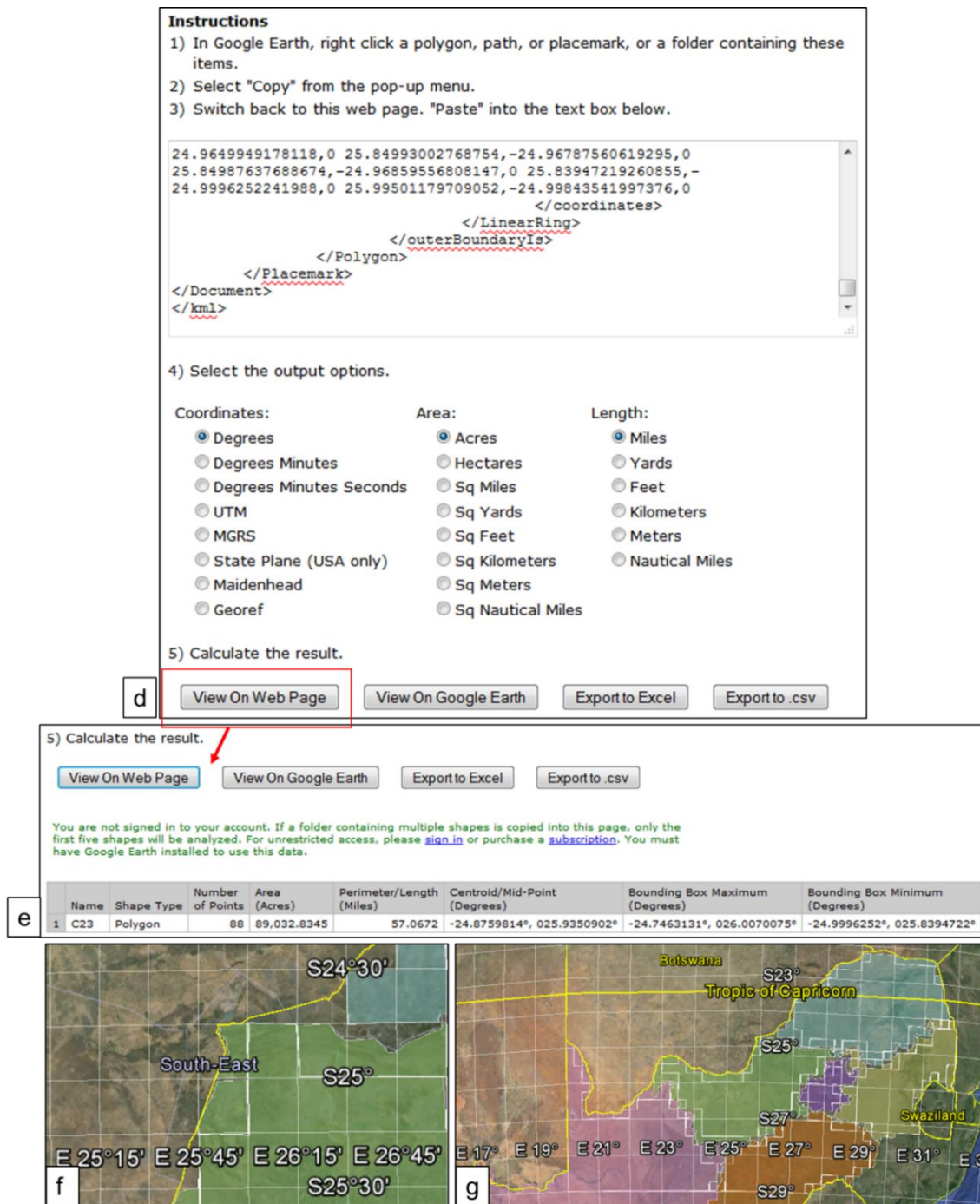


Figure 3.2 (cont.): The selected polygon area (d, e) was subtracted from the QDGC area, and if larger than the non-selected, was included in the study area (f). This method was applied to all QDGC in the study area and along borders of provinces (g).

3.3 Specimen data

Specimen data was obtained from 12 major herbaria throughout the study area. The herbaria are: A.P. Goossens Herbarium (PUC), Buffelskloof Private Nature Reserve Herbarium (BNRH), C.E. Moss Herbarium (J), Geo-Potts Herbarium (BLFU), H.G.W.J. Schweickerdt Herbarium (PRU), Kimberley South African National Parks Herbarium (KSAN), Larry Leach Herbarium (UNIN), McGregor Museum Herbarium (KMG), Mpumalanga Parks Board Herbarium (LYD), National Museum Herbarium (NMB), Pretoria National Herbarium (PRE), and UJ Department of Botany Herbarium (JRAU).

The use of herbarium specimen data to generate outcomes such as species richness and biogeographical regions, poses several potential limitations (Robertson & Barker 2006; De la Estrella *et al.* 2012). Sampling efforts may not be consistent, with some quarter degree grid cells (QDGC's) sampled excessively owing to geographical bias (along main roads or in nature reserves), taxonomic bias (species that are easy to collect or more conspicuous) and temporal bias (collected in one season). Other weaknesses include: 1) incorrect identification of specimens; 2) outdated taxonomy and 3) incorrect geo-referencing (Soberón & Peterson 2004).

Each specimen was assigned to a QDGC, either by coordinate data already available or determined from locality information on the label (Figure 3.3a). The precision of the collecting points varied, with more recent specimens typically having more precise location data available. Specimens with limited geographical data were assigned to the centre of the QDGC. For example, a specimen collected in the Johannesburg area would then have been assigned to the QDGC in the centre of Johannesburg.

Specimens were identified to species level when possible, and classified according to Al-Shehbaz & Warwick (2007), Fuentes-Soriana & Al-Shehbaz (2013), GRIN (2015), and Warwick *et al.* (2010) (Figure 3.3b, c). Species that were identified to genus level were considered additional species to the species list (Appendix B) when no species had been identified within the genera. Where multiple specimens had been identified to species level, it was assumed that the genus-specific specimen was one of the species identified. Data was sorted, and duplicate and cultivated specimen records were removed.

3.4 Centroid Grid profile

Data was standardized by using the Centroid Grid profile as described by Kurzweg (2011) to identify additional species that may be present in undersampled QDGC's as a means to determine the most likely distribution of the Brassicaceae throughout the study area. The study area was superimposed onto vegetation units of Mucina & Rutherford (2006) (Figure 3.4a). To simplify the standardization method, each QDGC was labelled with both its grid number (e.g. 2629BA) and all species that was recorded therein (Figure 3.4b, c).

Of the eight QDGC's surrounding a centroid grid (Figure 3.4c,d), the three grids with the most similar vegetation units to the centroid grid were identified, with additional consideration given to the dominant vegetation units. All the species of three chosen grids were then added to the centroid grid (Figure 3.4e). This was repeated for all the QDGCs in the study area to obtain the standardized data set (Appendix D). All the changes were recorded in a spreadsheet including which species were added to which QDGC (Figure 3.4f, g) (see Appendix D).

1	Species	GPS Location	GPS	Grid Ref	Location
2	<i>Alyssum minutum</i>			3118DD	Kaap
3	<i>Alyssum minutum</i>			3120CC	Northern C
4	<i>Arabidopsis thaliana</i>			2917DA	Namakwal
5	<i>Arabidopsis thaliana</i> (L.) Heynh.		29.99776 S 19.356995 E	2919CD	Cape Provi
6	<i>Arabidopsis thaliana</i> (L.) Heynh.		33°37'30"S 23°22'30"E	3323CB	Cape Provi
7	<i>Arabidopsis thaliana</i> (L.) Heynh.	Koue Bokkeveld:	33°04'59"S 19°25'00"E	3319AB	Cape Provi
8	<i>Arabidopsis thaliana</i> (L.) Heynh.		31.510362 S 24.98354 E	3124DB	Cape Provi
9	<i>Bornmuellera kiyakii</i> Aytag & Aksoy		37°23'30"N 31°30'33"E		C4 Konya; I
10	<i>Brachycarpaea juncea</i>			3118DD	Kaap; Gifbe
11	<i>Brachycarpaea juncea</i>			3118DD	Kaap; Gifbe
12	<i>Brachycarpaea juncea</i>			2917DB	Northern C
13	<i>Brachycarpaea juncea</i>			2917DA	Kaap; Sprin
14	<i>Brassica juncea</i> (L.) Czern. & Coss.		19°52'30"S 21°7'30"E	1921CC	Botswana;
a	<i>Brassica</i> sp.		30°8'0" S 23°31'0" E	3023BA	Hay; Nieuw
	<i>Brassica</i> sp.	Thornley farmstead	29°11'0" S 23°17'0" E	2923AB	Hay; Thorn

1	Species	(Is) Species	Genera	#	n species	Grid Ref
2	<i>Alyssum</i> sp.	<i>Alyssum</i> sp.	<i>Alyssum</i>	1	1	2628AA
3	<i>Brachycarpaea juncea</i>	<i>Heliophila juncea</i> (P.J. Bergius) Druce		0		2917DA
4	<i>Brachycarpaea juncea</i>	<i>Heliophila juncea</i> (P.J. Bergius) Druce		0		2917DA
5	<i>Brachycarpaea juncea</i>	<i>Heliophila juncea</i> (P.J. Bergius) Druce		0		2917DA
6	<i>Brassica elongata</i> Ehrh. ssp. <i>elongata</i>	<i>Brassica elongata</i> Ehrh. ssp. <i>elongata</i>	<i>Brassica</i>	3	6	2829AD
7	<i>Brassica elongata</i> Ehrh. ssp. <i>elongata</i>	<i>Brassica elongata</i> Ehrh. ssp. <i>elongata</i>		0		2525CC
8	<i>Brassica elongata</i> Ehrh. ssp. <i>elongata</i>	<i>Brassica elongata</i> Ehrh. ssp. <i>elongata</i>		0		2327AD
9	<i>Brassica elongata</i> Ehrh. ssp. <i>elongata</i>	<i>Brassica elongata</i> Ehrh. ssp. <i>elongata</i>		0		2229AC
10	<i>Brassica elongata</i> Ehrh. ssp. <i>elongata</i>	<i>Brassica elongata</i> Ehrh. ssp. <i>elongata</i>		0		2426BB
11	<i>Brassica juncea</i> (L.) Czern. & Coss.	<i>Brassica juncea</i> (L.) Czern. & Coss.		0		2329BB
12	<i>Brassica juncea</i> (L.) Czern. & Coss.	<i>Brassica juncea</i> (L.) Czern. & Coss.		0		2827DD
13	<i>Brassica juncea</i> (L.) Czern. & Coss.	<i>Brassica juncea</i> (L.) Czern. & Coss.		0		2826AC
14	<i>Brassica juncea</i> (L.) Czern. & Coss.	<i>Brassica juncea</i> (L.) Czern. & Coss.		0		2230CD
b	<i>Brassica juncea</i> (L.) Czern. & Coss.	<i>Brassica juncea</i> (L.) Czern. & Coss.		0		2626CC
	<i>Brassica nigra</i> (L.) W.D.J.Koch	<i>Brassica nigra</i> (L.) W.D.J.Koch		0		2530BD

1	(Is) Species	Genera	#	n species	Grid Ref
2	<i>Alyssum</i> sp.	<i>Alyssum</i>	1	1	2628AA
3	<i>Armoracia rusticana</i> P. Gaertn., B. Mey. & Scherb.	<i>Armoracia</i>	2	1	2628AA
4	<i>Brassica elongata</i> Ehrh. ssp. <i>elongata</i>	<i>Brassica</i>	3	5	2829AD
5	<i>Brassica elongata</i> Ehrh. ssp. <i>elongata</i>		0		2525CC
6	<i>Brassica elongata</i> Ehrh. ssp. <i>elongata</i>		0		2327AD
7	<i>Brassica elongata</i> Ehrh. ssp. <i>elongata</i>		0		2229AC
8	<i>Brassica elongata</i> Ehrh. ssp. <i>elongata</i>		0		2426BB
9	<i>Brassica juncea</i> (L.) Czern. & Coss.		0		2329BB
10	<i>Brassica juncea</i> (L.) Czern. & Coss.		0		2827DD
11	<i>Brassica juncea</i> (L.) Czern. & Coss.		0		2826AC
12	<i>Brassica juncea</i> (L.) Czern. & Coss.		0		2230CD
13	<i>Brassica juncea</i> (L.) Czern. & Coss.		0		2626CC
14	<i>Brassica nigra</i> (L.) W.D.J.Koch		0		2530BD
c	<i>Brassica nigra</i> Koch		0		2627CA
	<i>Brassica rapa</i>		0		2229AB

Figure 3.3: Assigning a single QDGC to each specimen (a) and classifying each species correctly (b) to obtain the final data set (c) in Microsoft Office: Excel 2013.

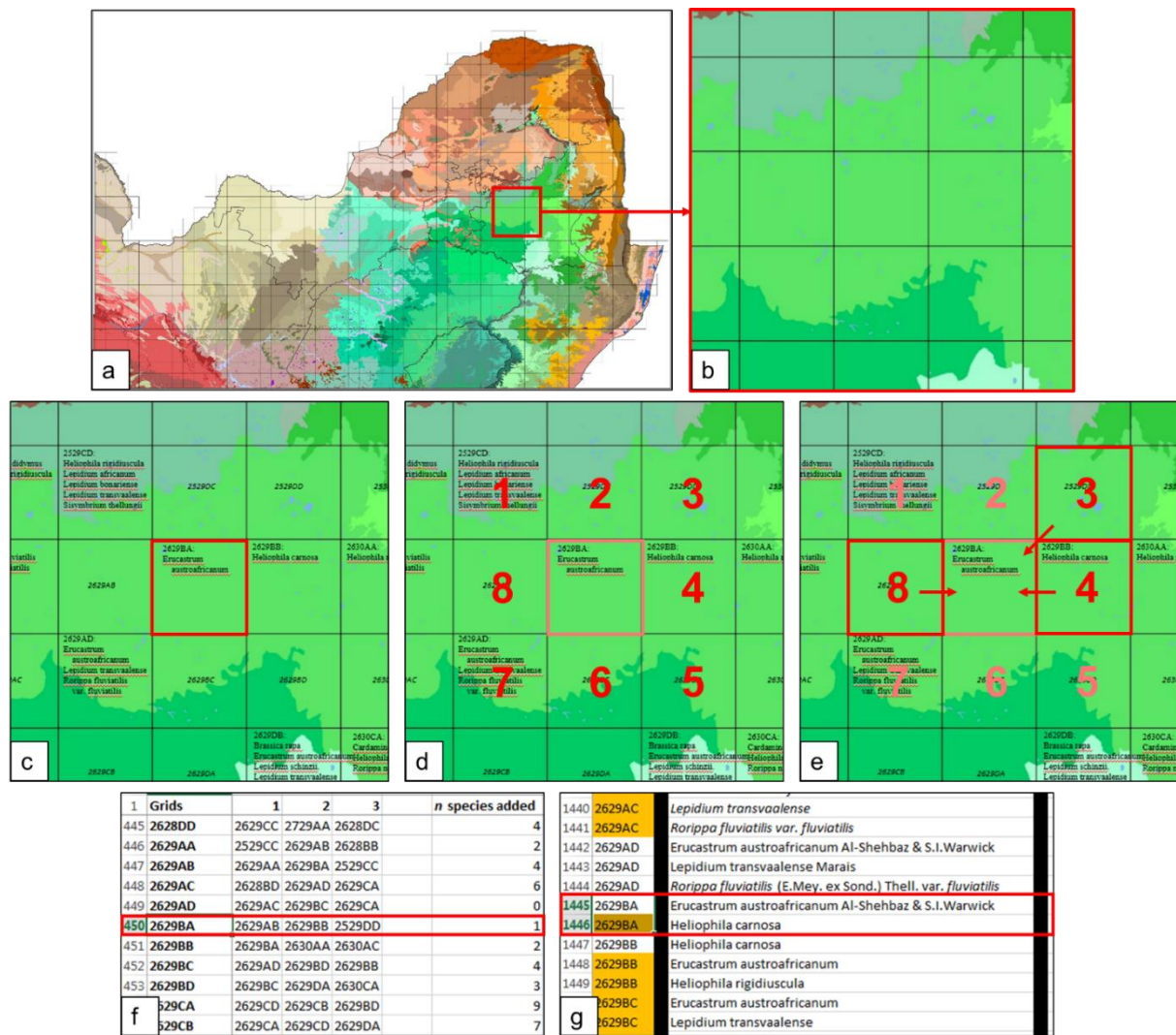


Figure 3.4: Vegetation units described by Mucina & Rutherford (2006) (a); eight QDGC's surrounding centroid grid (b); centroid grid with red borders (c); eight surrounding QDGCs numbered (d); species additions from three surrounding grids with the most similar vegetation units (e); spreadsheet indicating the three selected grids and the number of species to be added to 2629BA (f); spreadsheet indicating the three selected grids and the number of species to be added to 2629BA (g).

Further information regarding the use of Centroid Grid profile for standardization is provided in Chapter 4.2.3.

3.5 Data analyses

Both non-standardized and standardized (Centroid Grid profile) data was divided into three data sets for analysis: species, genera and tribes (Figure 3.5). Each data set was transformed to a matrix for use in PRIMER and PAST (Figure 3.5).

	A	B	C	D
1	SPP	2229AC	2229CC	2229DD
2	<i>Alysp.</i>	0	0	0
3	<i>Armrus</i>	0	0	0
4	<i>Braelo</i>	1	0	0
5	<i>Brajun</i>	0	0	0
6	<i>Branig</i>	0	0	0
7	<i>Brarap</i>	0	1	0
8	<i>Braraps</i>	0	0	0
9	<i>Brasp.</i>	0	0	0
10	<i>Camrum</i>	0	0	0
11	<i>Capbur</i>	0	0	0
12	<i>Carafra</i>	0	0	0

	A	B	C	D
1	GENERA	2229AC	2229CC	2229DD
2	<i>Alyssum</i>	0	0	0
3	<i>Armoracia</i>	0	0	0
4	<i>Brassica</i>	1	1	0
5	<i>Camelina</i>	0	0	0
6	<i>Capsella</i>	0	0	0
7	<i>Cardamine</i>	0	0	0
8	<i>Coronopus</i>	0	0	0
9	<i>Descurainia</i>	0	0	0
10	<i>Diplotaxis</i>	0	0	0
11	<i>Eruca</i>	0	0	0
12	<i>Erucastrum</i>	1	0	0

	A	B	C	D
1	TRIBE	2229AC	2229CC	2229DD
2	Alysseae	0	0	0
3	Anchonieae	0	0	0
4	Brassicaceae	2	1	0
5	Camelineae	0	0	0
6	Cardamineae	0	0	1
7	Descurainieae	0	0	0
8	Erysimeae	0	0	0
9	Heliophilleae	0	0	0
10	Lepidieae	1	0	0
11	Sisymbrieae	0	0	0
12				

Figure 3.5: Non-standardized species, genera and tribe data sets for use in PRIMER and PAST. The presence of species was indicated with a “1” and absence with a “0”. Genera and tribes show the number of species present in each genus and tribe respectively, with absence indicated with a “0”.

3.5.1 PRIMER

PRIMER software (version 6) (PRIMER-E 2012) was used. The data was standardized using square root as pre-treatment and the Bray-Curtis similarity index was applied. Initial non-metric multidimensional scaling (NMDS) graphs were obtained to identify possible outliers (Figure 3.6). Dendrograms were constructed from Bray-Curtis similarity results to indicate clusters of samples with the most similar species composition. Similarity profile (SIMPROF) was used to indicate definite objective clusters (Clarke & Gorley 2005) (Figure 3.7). A resemblance slice (subjectively chosen according to the results obtained, with the highest being at 50% similarity and lowest at 5%) was applied to indicate clusters. When a cluster was defined as definite by SIMPROF, all including dendrogram branches were traced to the first definite junction and used as a single cluster.

NMDS ordinations were used for final data analysis. Clusters, indicated by dendrograms in conjunction with SIMPROF, were inserted into NMDS using Factors (Figure 3.8).

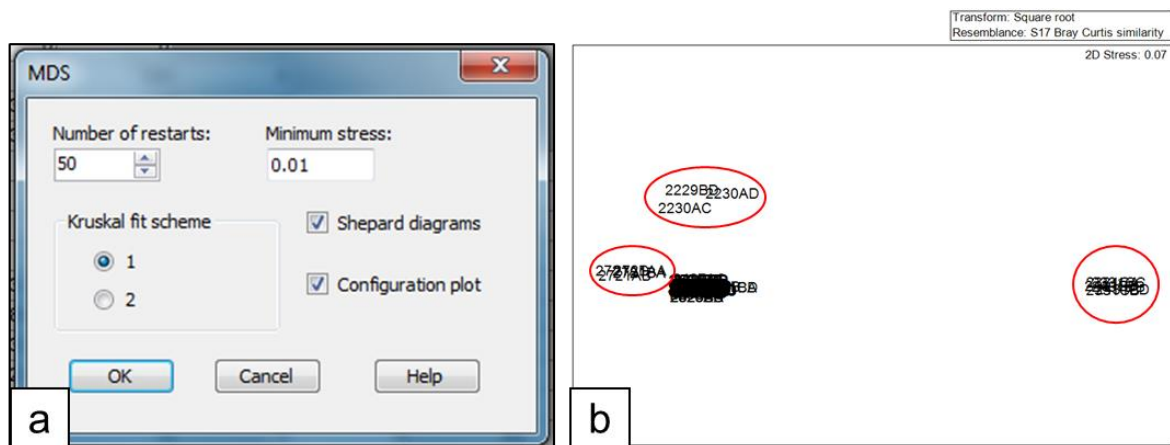


Figure 3.6: Non-metric multidimensional scaling (NMDS) ordinations were applied to each of the data sets (a). Outlier QDGC's were removed where needed (b).

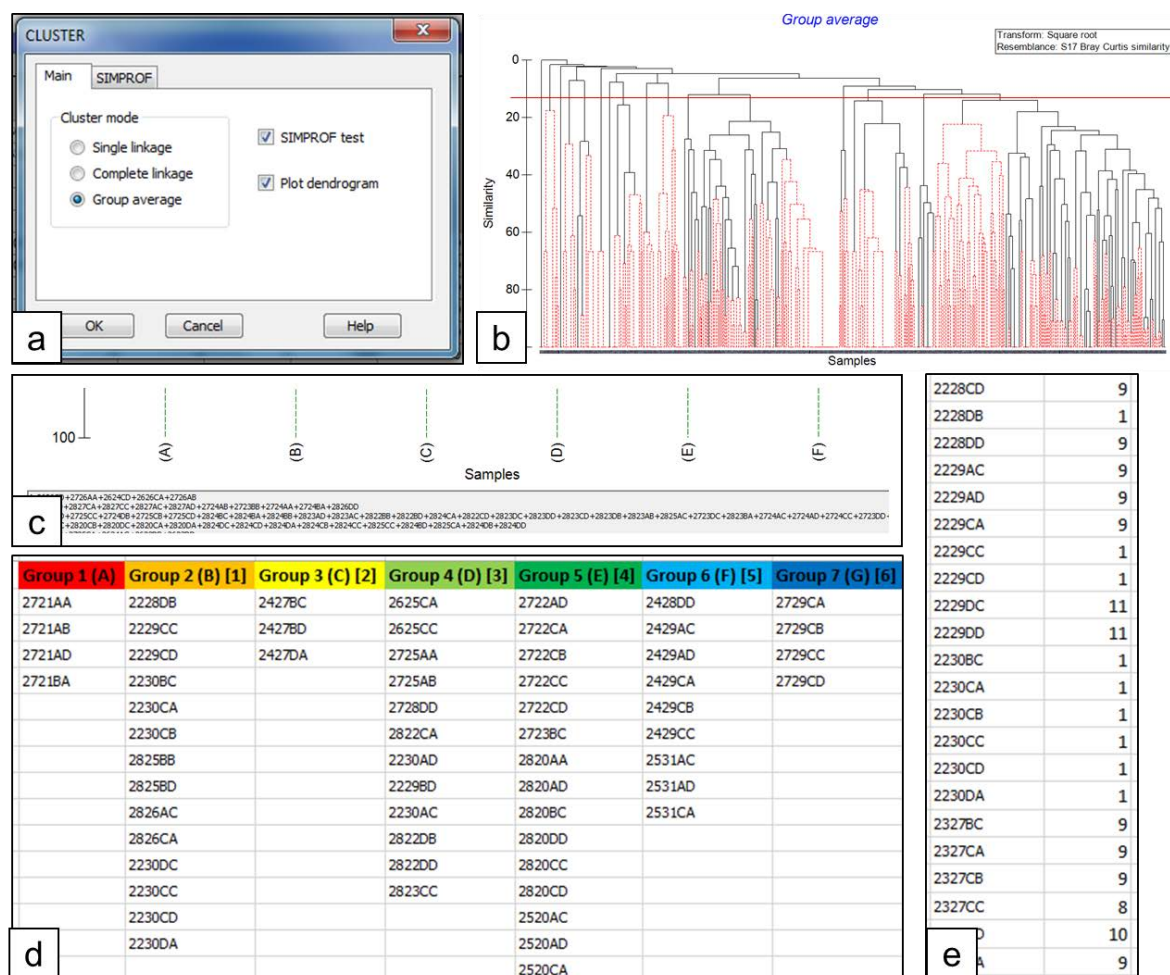


Figure 3.7: Similarity profiles (SIMPROF) were included in every dendrogram (a). Solid black lines indicate definite objective clusters (b). No more than 15 clusters were identified in each data set with the use of a cut-off line (c). The QDGC's belonging to each cluster were identified (d). Each QDGC was numbered according to cluster and sorted (e) for further use of species data in PRIMER and PAST.

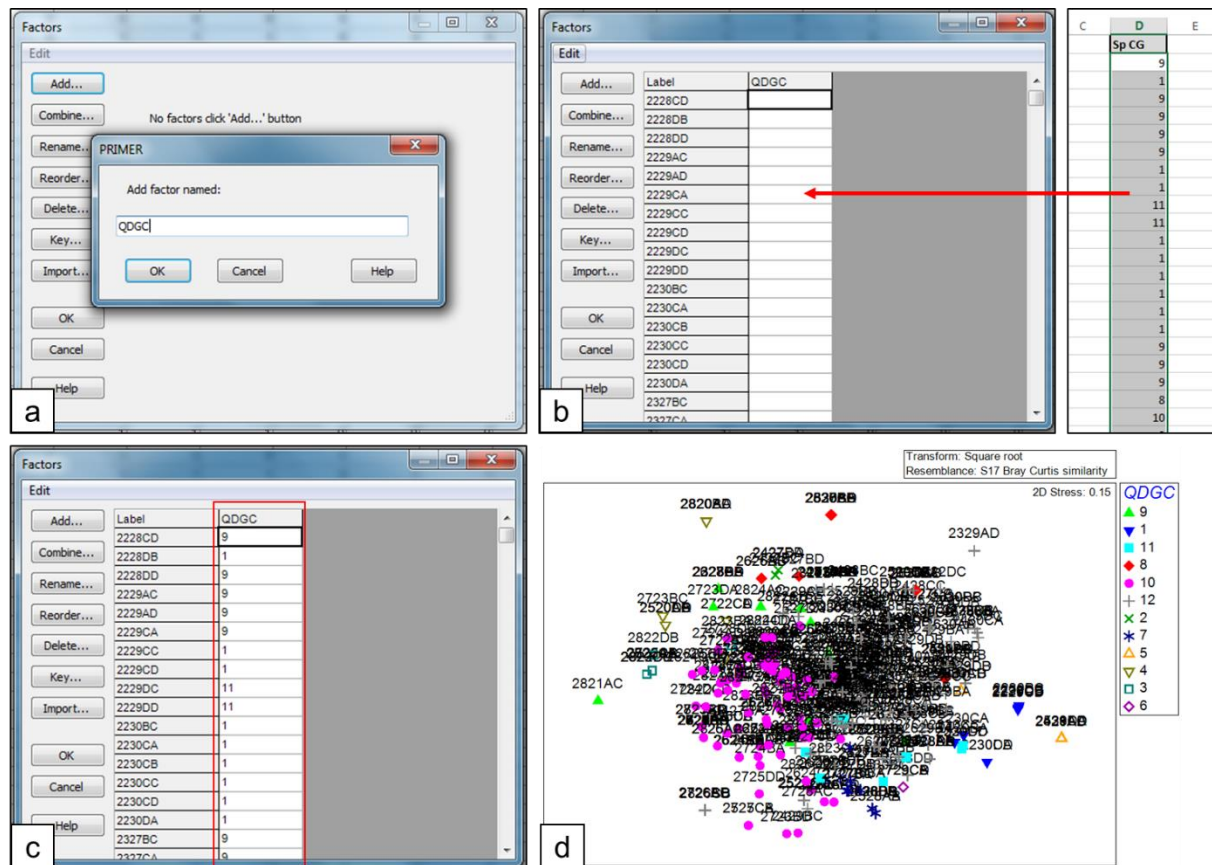


Figure 3.8: The improved data set (without outliers) was analysed with PRIMER, and factors added (a). The samples were imported into the “factor” created (b). These settings ensured the creation of clusters as specified (c). Fourth-root transformation and Bray-Curtis similarity index was applied, followed by NMDS ordination that now include clusters (d).

3.5.2 PAST

Each of the six data sets (standardized/non-standardized species, genera and tribes) was imported into Paleontological Statistics program software (PAST) (Figure 3.9a–c). The six data sets were analysed for similarities (ANOSIM) between clusters as determined by dendrogram (Figure 3.9d) and similarity profiling (SIMPROF) (Figure 3.9e, f). The values obtained (p , R and similarity contribution %) were placed into a table for further analysis in Chapter 4.

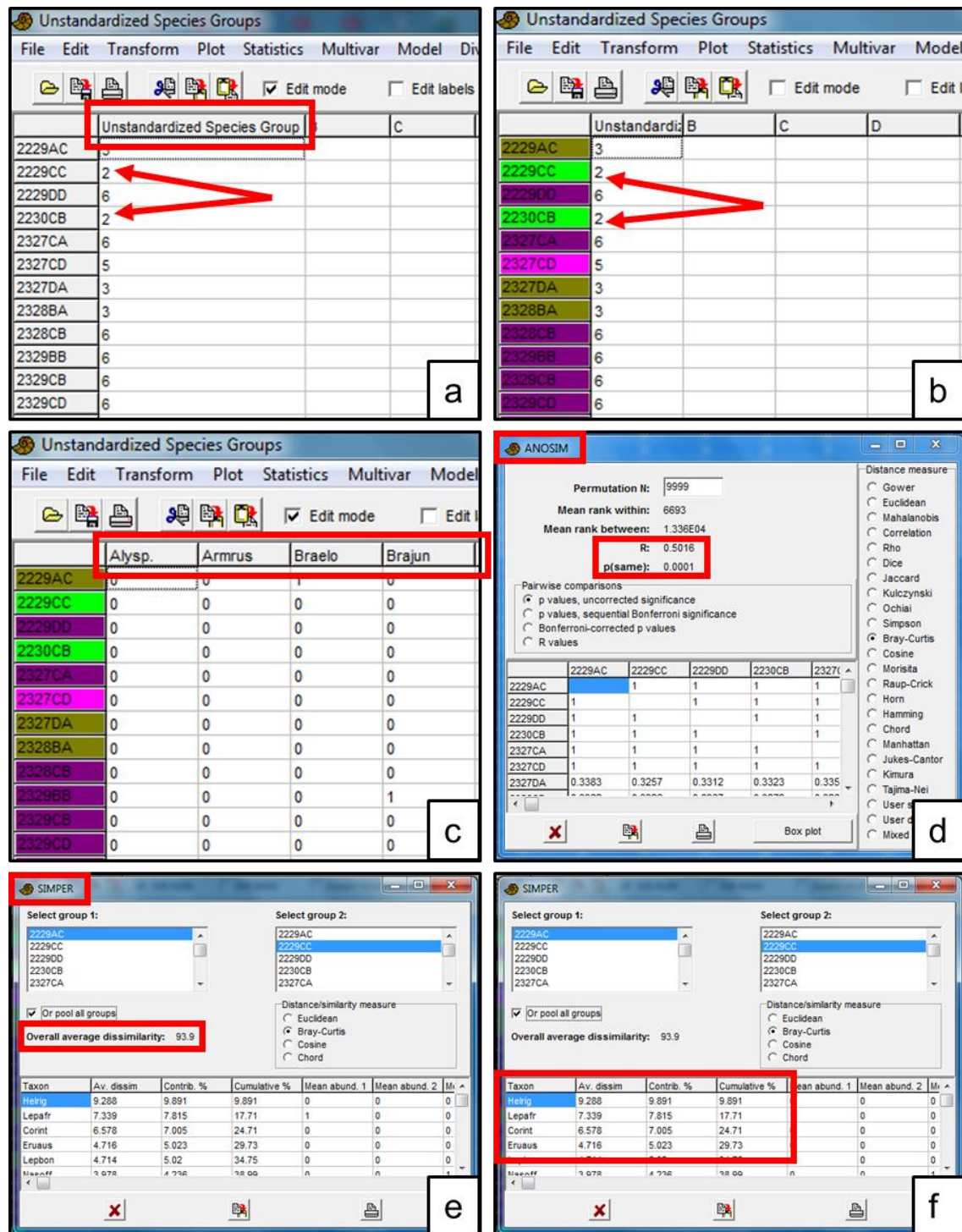


Figure 3.9: All QDGC's were imported into PAST with cluster numbers according to Bray-Curtis and SIMPROF (a). The QDGC's were clustered according to cluster numbers (arrows, b) by using the "Numbers to colors/symbols" function. The data set data was manipulated to create subsets for species, genus or tribe (c). ANOSIM was applied to obtain R and *p* values (d) and SIMPER for average dissimilarity per data set (e). The species, genera and tribes that contributed the most to the distribution patterns of the Brassicaceae were identified (f).

3.6 Map layers

Each of the six data set distributions was plotted on a map to visually indicate the distribution patterns of the clusters obtained. The three standardized data sets (species, genus and tribe) were subjectively altered as layers for further stacking purposes. The standardized species clusters are provided as example. Each QDGC was coloured according to the clusters identified (as seen in Chapter 5) (Figure 3.10).

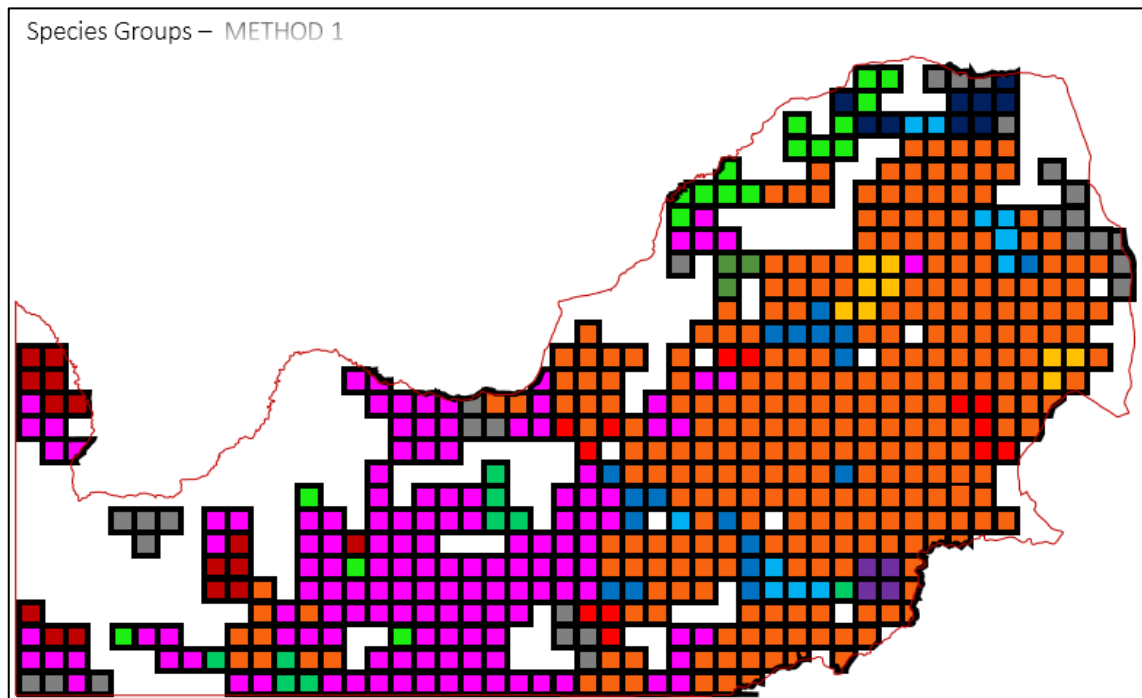


Figure 3.10: Standardized species QDGC's coloured according to clusters as determined by SIMPROF.

Two different methods were compared to determine which would work best when the distribution map was stacked with different rainfall maps. The first method did not change the borders surrounding the QDGC's (Figure 3.11). A transparency of 50% was applied for later use. In the second method, each cluster was subjectively marked (Figure 3.12, 3.13). Each of these newly marked clusters were further defined, and a transparency of 50% was applied (Figure 3.14).

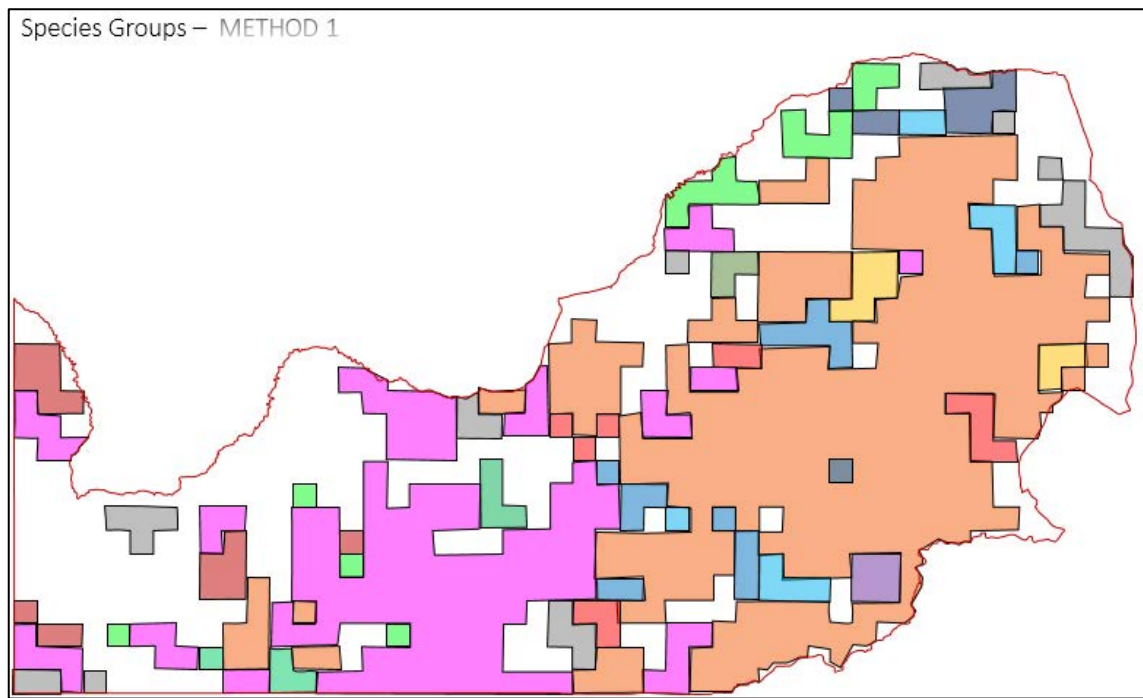


Figure 3.11: Method 1 with the standardized species clusters with similar species composition, as determined by Bray-Curtis and dendrogram, showing the study area (outlined in red), with a transparency of 50% applied.

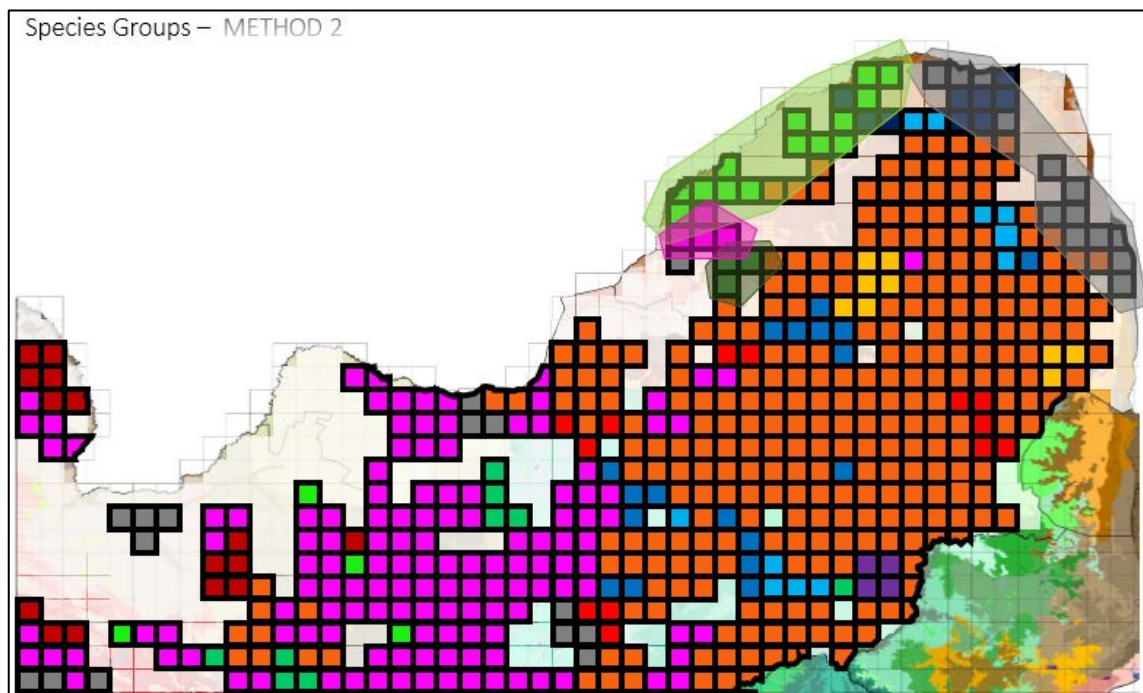


Figure 3.12: Method 2 involved the clusters being subjectively marked. The example of the first large grey and green areas are indicated.

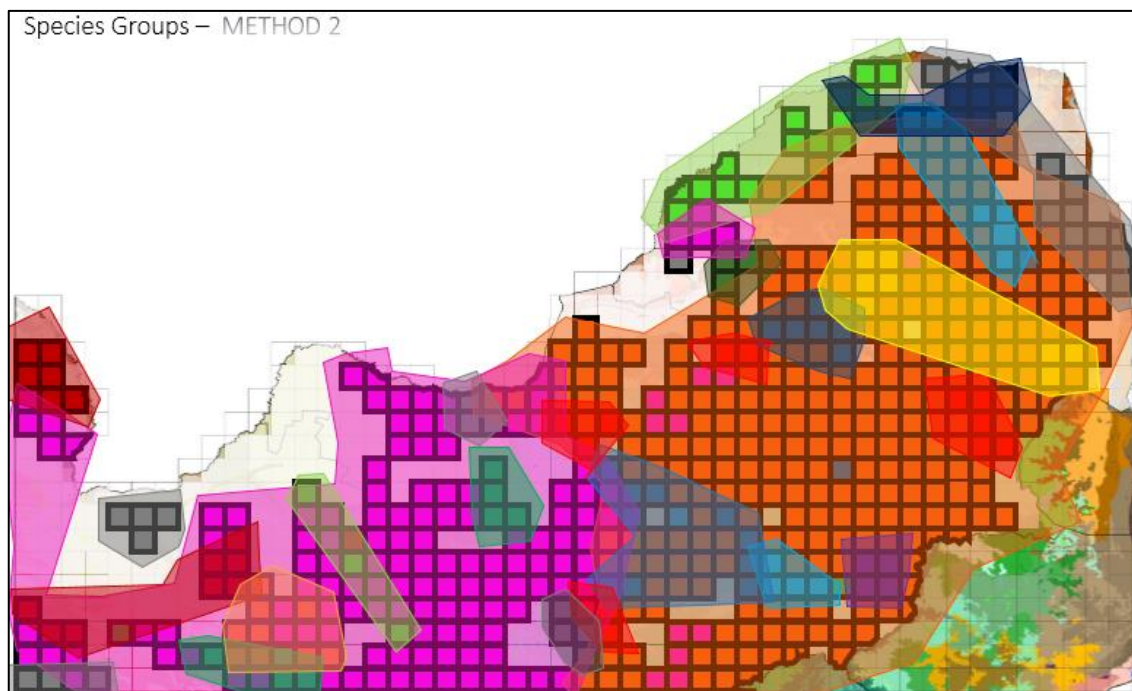


Figure 3.13: Method 2 with all QDGC's that seemed to form clusters being placed into subjectively chosen areas.

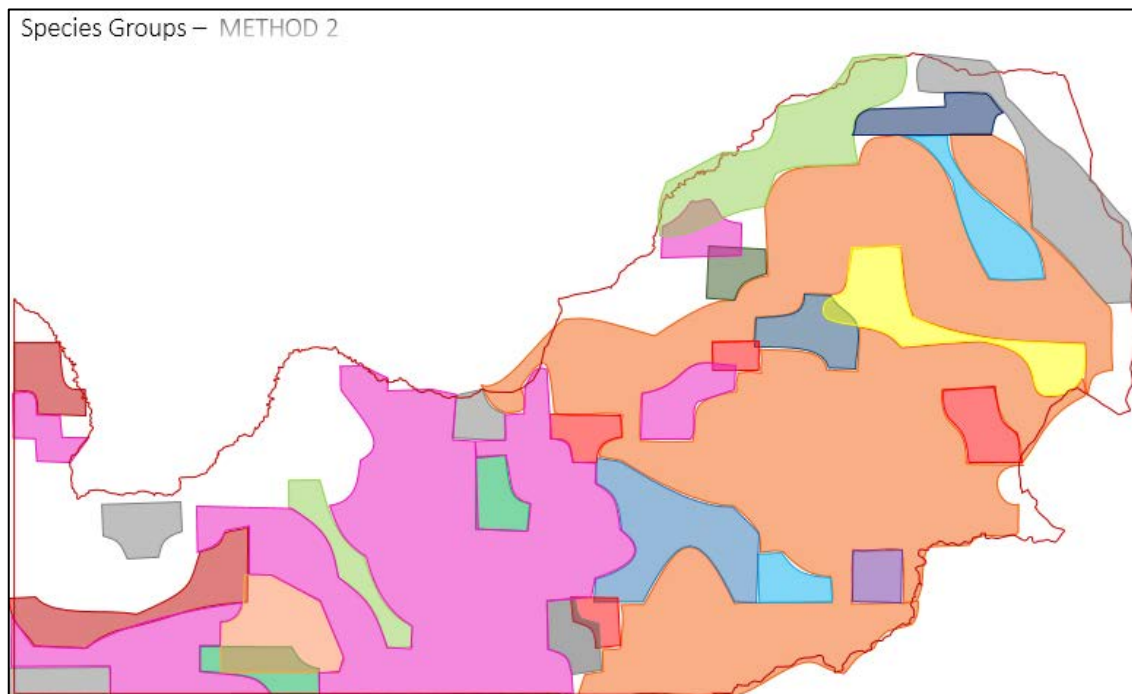


Figure 3.14: Method 2 with the standardized species clusters subjectively marked, showing the study area (outlined in red), with a transparency of 50% applied.

The second method was chosen to stack with different rainfall maps to reduce the amount of numbers that will be added to indicate which clusters are present in which locations. The stacking of maps provided a means to find possible explanations for the distribution patterns of the Brassicaceae. Base maps included the bioregions of Mucina & Rutherford (2006) (Figure 3.15), which was adapted to include only the bioregions present within the study area (Figure 3.16). Secondly, the non-standardized and standardized Brassicaceae were overlapped with rainfall maps: seasonal rainfall indicating amount of rainfall (in mm) from July 2014 to January 2015 was retrieved from the South African Weather Service website (SAWS 2015) (Figure 3.17), and mean annual rainfall over the course of 50 years (1950–1999) was compiled by Lynch (2004) (Figure 3.18). The seasonal period (July to January; Figure 3.17) was used due to the fact that *Brassica napus* is a winter crop, and the long term rainfall chart to consider the time frame in which the specimens used for analyses had been collected.

The distribution of the Brassicaceae may be correlated with collection efforts along roads. Transport route maps were stacked with the standardized distribution obtained for species, genera and tribes. During the risk assessment (see Chapter 5), QDGC's were marked according to the presence of reproductively compatible species. This map of reproductively compatible species was stacked with a map indicating main roads, cities and towns (United Nations 2007). The roads, cities and towns were identified and clearly marked (Figure 3.19).

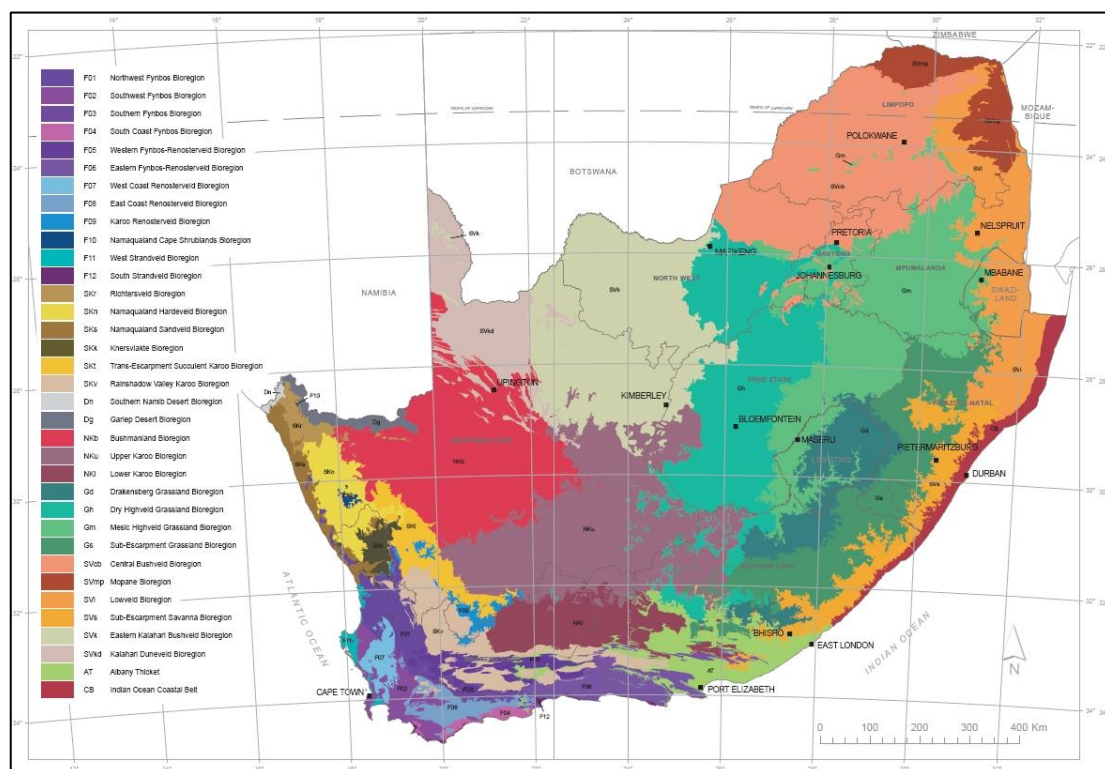


Figure 3.15: Bioregions of South Africa (Mucina and Rutherford, 2006).

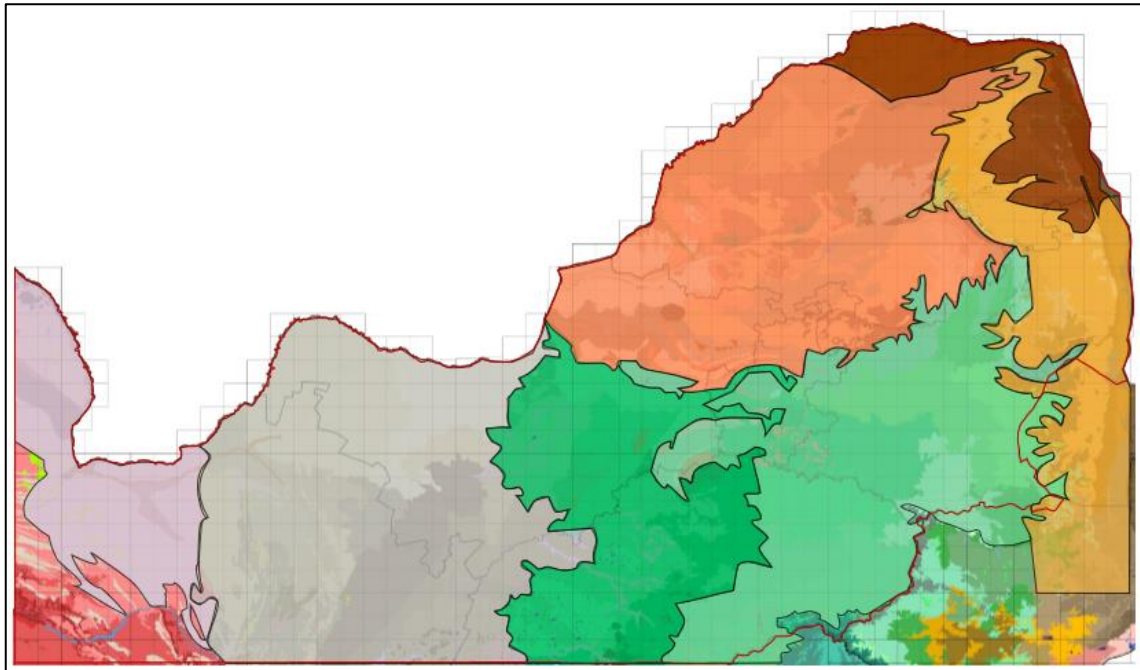


Figure 3.16: Bioregions of South Africa (Mucina & Rutherford 2006), adapted to include only the regions present in the area of study. The map it is overlaid onto is the vegetation units of South Africa (Mucina & Rutherford 2006).

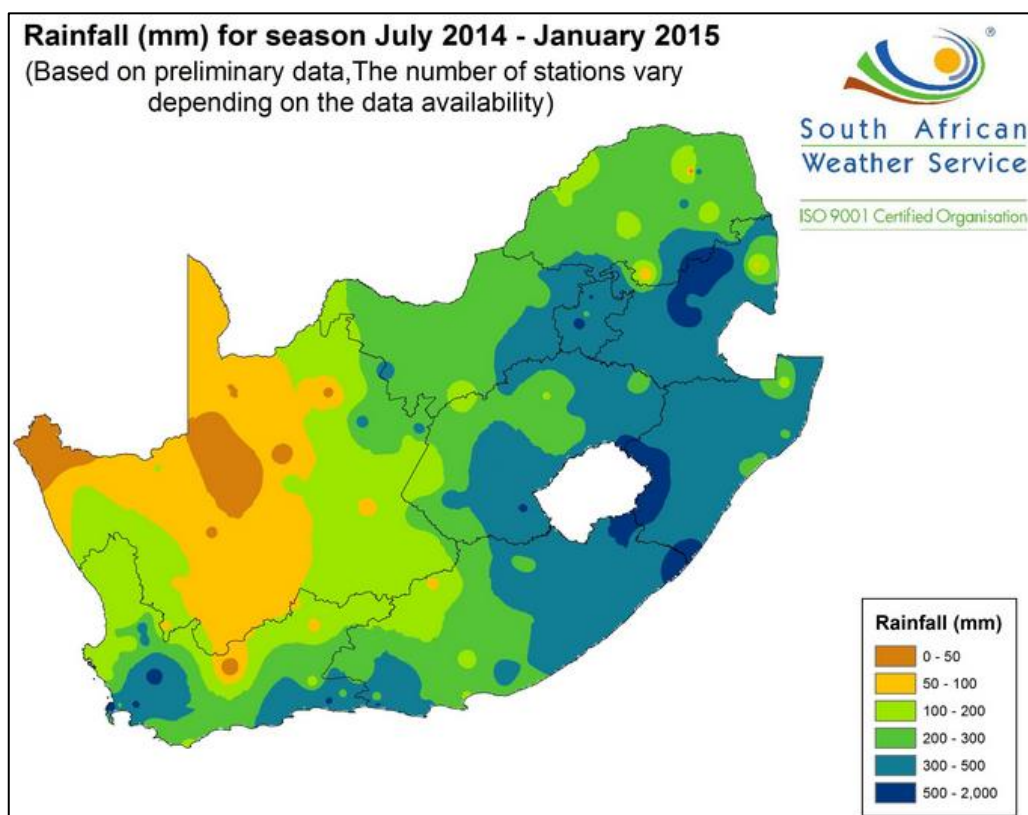


Figure 3.17: Map indicating seasonal rainfall during July 2014 to January 2015, obtained from SAWS (2015).

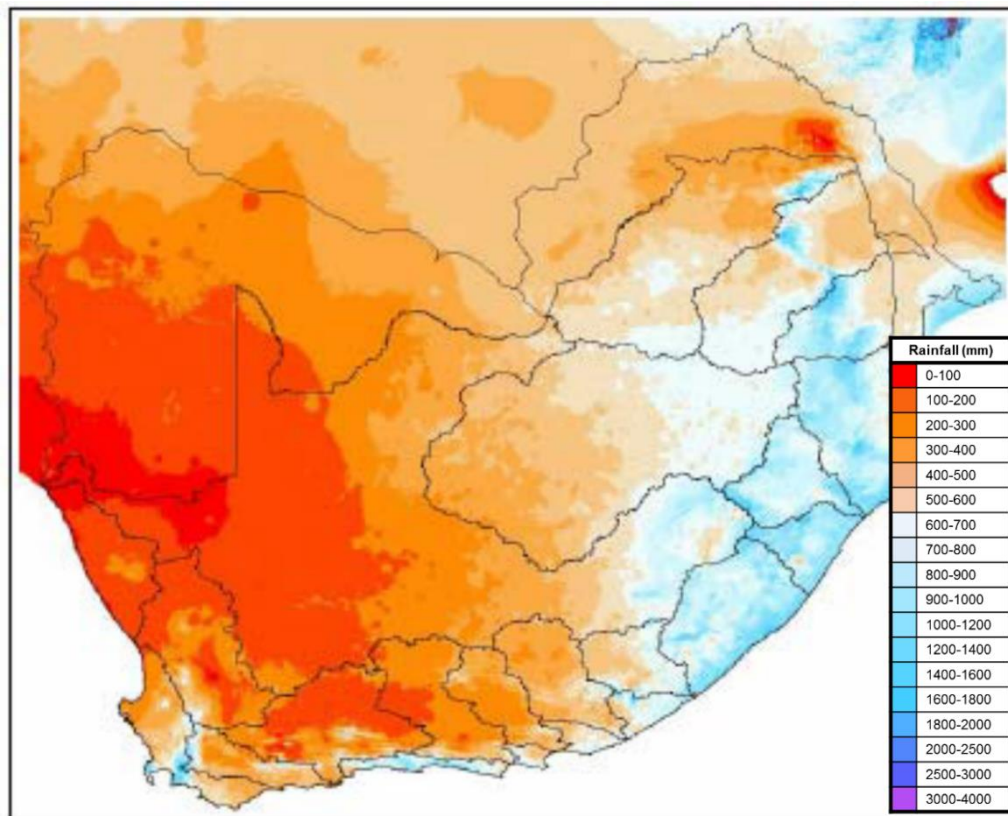


Figure 3.18: Mean rainfall between 1950 and 1999 (Lynch 2004).



Figure 3.19: Map of South Africa indicating major roads, cities, towns, and railroads. Obtained from United Nations (2007).

3.8 References

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Chapter 4: Phytogeography of the Brassicaceae

4.1 Introduction

The Brassicaceae is widely distributed throughout southern Africa (Glen 2002; Glen & Jordaan 2003; Retief & Herman 1997; Warwick *et al.* 2003) in the wild or as crops (Schoenenberger & D'Andrea 2012). *Brassica napus*, otherwise known as rapeseed, oilseed rape or canola, is the third most planted oil crop in South Africa, with 95 000 ha planted in the winter of 2014 (Dredge 2015; Légère 2005). The seed of *B. napus* is commercially used for the production of crude canola oil, canola biodiesel, canola meal (animal feed and fertilizer), and canola oil (canola based mayonnaise, oil cake, cooking oil, margarine and salad dressing) (DAFF 2013).

Considerable work has been done on gene flow within the Brassicaceae, especially between crops and their wild and weedy relatives (FitzJohn *et al.* 2007; Hall *et al.* 2000; Stewart *et al.* 1997; Warwick *et al.* 2003). Gene flow plays an important role in the potential evolution and invasiveness of species (Barbour *et al.* 2010; Ellstrand & Schierenbeck 2000). Hybridization between *B. napus* and its wild and weedy relatives may cause multiple changes in the F₁ hybrids, such as the transferral of beneficial genes, an increase in genetic diversity and increased colonization and dispersal (Barbour *et al.* 2010; Hauser *et al.* 1998; Stewart *et al.* 2003). Gene flow may occur between reproductively compatible species via the distribution of seeds, volunteers or pollen flow (Aono *et al.* 2006; Ceddia *et al.* 2009; FitzJohn *et al.* 2007; Gulden *et al.* 2003; Knispel *et al.* 2008; Levin & Kerster 1974). Pollen flow may occur via wind or insects that may lead to self-pollination or cross-pollination (Aono *et al.* 2006; Levin & Kerster 1974).

The distribution of *B. napus* by seed dispersal may occur via anthropological activities or environmental factors (Barnaud *et al.* 2013; Gulden *et al.* 2003; Schoenenberger & D'Andrea 2012). Seed may be distributed anthropologically via transportation by railway lines (Schoenenberger & D'Andrea 2012), roads (Barnaud *et al.* 2013; Crawley & Brown 1995; Pessel *et al.* 2001) and other methods such as when dropping from harvesting equipment (Senior & Dale 2002) and fireworks (Schoenenberger & D'Andrea 2012). *Brassica napus* seeds may naturally be distributed by animals such as birds, mammals (Schoenenberger & D'Andrea 2012) and insects (Levin & Kerster 1974; Smyth *et al.* 2002), and abiotic factors such as wind (Levin & Kerster 1974; Smyth *et al.* 2002). Seeds of *B. napus* may form volunteer populations in the seedbank that may germinate after extended periods of time (Beckie *et al.* 2003; Pessel *et al.* 2001; Reagon & Snow 2006; Warwick *et al.* 2003).

Volunteer populations may act as genetic bridge species. Bridge species are frequently found between different varieties of a species known to hybridize with its relatives (Hall *et al.* 2000). In a study by Reagon & Snow (2006), transgene flow occurred from cultivated *Helianthus annuus* L. (sunflower) to common volunteer wild sunflowers (also *H. annuus*) that acted as a genetic bridge (Reagon & Snow 2006). When volunteers act as genetic bridges the temporal and distribution ranges in which gene flow may occur increases (Beckie *et al.* 2003; Hall *et al.* 2000; Reagon & Snow 2006). Furthermore, even when the crop species is harvested or removed, the volunteer population may continue transferring the transgene to reproductively compatible relatives – as often occurs in crops that are harvested before flowering time such as beet and lettuce (Reagon & Snow 2006).

Herbicide tolerant *B. napus* was cultivated and have been available in Canada since 1982 (Senior & Dale 2002), but genetically modified *B. napus* (herbicide tolerant) was first commercialized during 1995 in Canada (Simard *et al.* 2002). Transgenic varieties of *B. napus* were commercially planted in thirteen countries throughout the world, including Australia, Canada, Japan, and the United States of America, but is not cultivated in South Africa (Aono *et al.* 2006; Beckie *et al.* 2003; Rieger *et al.* 2002; Schafer *et al.* 2011). It is known that hybridization may occur between herbicide tolerant *B. napus* and its wild and weedy relatives such as *B. rapa*, *Raphanus raphanistrum* L., *Sinapis arvensis* L., and *Erucastrum gallicum* (Willd.) O.E. Schulz when the distributions of these species overlap (FitzJohn *et al.* 2007; Warwick *et al.* 2003). Hence, an overlap of the geographical distribution of *B. napus* and its wild and weedy relatives presents an opportunity for the potential evolution of transgenic traits into the environment (Barnaud *et al.* 2013).

The factors that influence the likelihood and establishment of gene flow and its potential negative consequences need to be determined to accurately assess the risk involved with the introduction and gene flow potential of transgenic *B. napus* to the northern provinces of South Africa (Andow & Hilbeck 2004; McGeoch *et al.* 2009). This spatial assessment endeavours to contribute partly to the risk assessment (Chapter 5) process by determining the likelihood of gene flow to occur based on sympatric distributions. This includes determining the distribution of the Brassicaceae, with special emphasis on the species that are reproductively compatible with *B. napus*, as a means to determine the potential for spatial boundaries to be crossed in order for gene flow to occur. Factors influencing the distribution of the Brassicaceae needs to be, and will be, taken into account.

A spatial assessment of the gene flow potential between *B. napus* and its wild and weedy relatives in South Africa was done by McGeoch *et al.* (2009), but was limited to the Fynbos Biome in the Western Cape. Even though the vegetation units present in the Fynbos Biome

differ from those present throughout the area of the current study (Mucina & Rutherford 2006), several of the Brassicaceae species present in the Fynbos also occurs in the northern provinces. The quantitative spatial risk assessment done by McGeoch *et al.* (2009) is based on the relative prevalence of a species, whether the spatial distributions overlap and whether it is a reproductively compatible species, or if unknown, whether the genus is known to be reproductively compatible with *B. napus*. Reproductively compatible species that have overlapping spatial distributions have overcome physiological and spatial barriers, increasing the gene flow risk associated between these species considerably.

Brassica napus is known to form fertile hybrids when hybridization occurs with wild and weedy relatives (Légère 2005; McGeoch *et al.* 2009; Stewart *et al.* 2003). Because of various reports from across the world where transgene movement has occurred from *B. napus* to wild and weedy relative populations in Canada (Beckie *et al.* 2003), England and Wales (Allainguillaume *et al.* 2006), and the United States (Schafer *et al.* 2011), it is important to quantify the diversity of wild and weedy *B. napus* relatives in northern South Africa and assess the spatial overlap of their distribution as a basis for understanding the potential for gene flow to occur from commercially produced *B. napus* to its reproductively compatible relatives. Although *Brassica napus* is not currently cultivated in northern South Africa (it has been cultivated on small scale for several years until approximately 2012). This chapter presents a determination of the spatial distribution of reproductively compatible species compared to potential cultivation areas of *B. napus*, and is a means to identify areas of distribution overlap that may be at highest risk for gene flow to occur.

4.2 Materials and methods

4.2.1 Literature study

A literature study was conducted to determine which relatives of *B. napus* were present in the northern provinces of South Africa. The study specifically targeted plant taxa that have the potential to hybridize with *B. napus*. Nomenclature follows Al-Shehbaz *et al.* (2006), APG III (2009) and Warwick *et al.* (2010). The Free State and Northern Cape provinces were only partially included in the study area to cover the parts containing target species (Mucina & Rutherford 2006) (Figure 4.1). KwaZulu-Natal was not included in the study area due to its sub-tropical climate and subsequent rarity of target species (Mucina & Rutherford 2006).

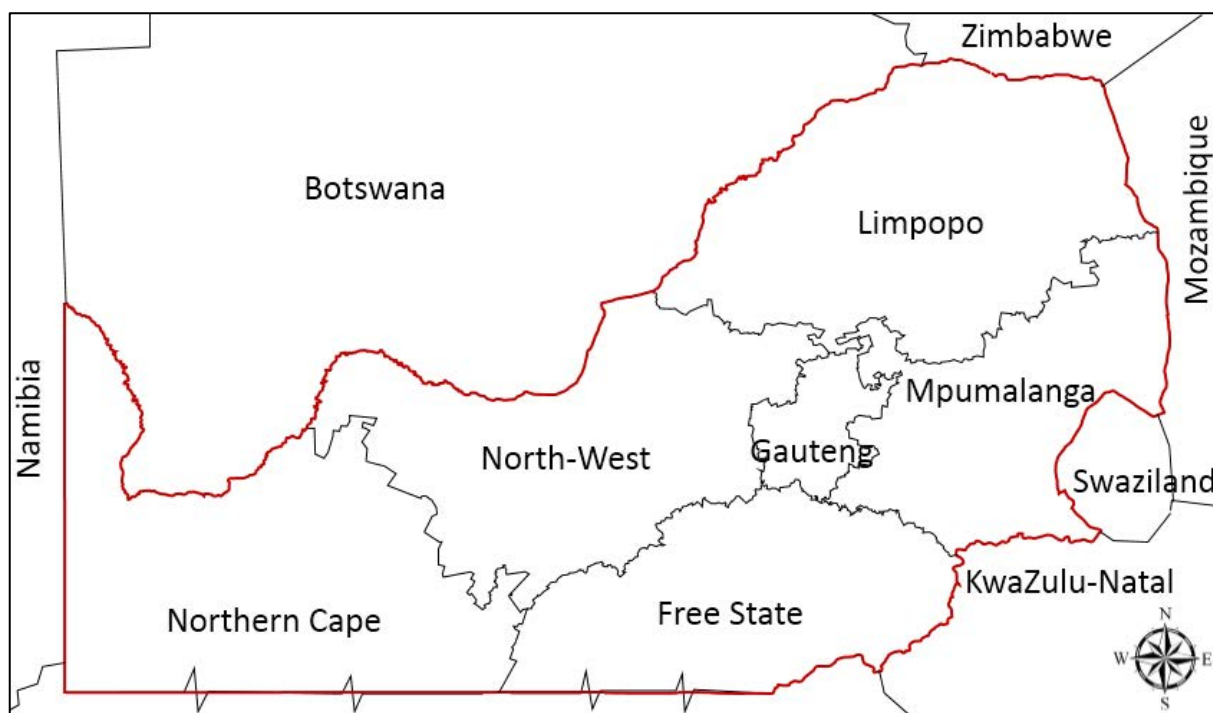


Figure 4.1: Study area (outlined in red) and the six northern provinces of South Africa included in the study.

4.2.2 Data processing

The study area was divided into quarter-degree grid cells (QDGC) of $\pm 692.5 \text{ km}^2$ per grid (see Chapter 3.2). Point distribution data for species were obtained from various major herbaria within the study area. All identified specimens of the Brassicaceae were considered. Specimen data was sorted according to species, genera and tribes respectively, duplicate and cultivated garden specimen records were removed, and species were correctly classified according to Al-Shehbaz & Warwick (2007), Fuentes-Soriana & Al-Shehbaz (2013), GRIN (2015), and Warwick *et al.* (2010) (see Chapter 3.3).

Various sources report 165 Brassicaceae species to occur in South Africa (Al-Shehbaz *et al.* 2006; Glen & Jordaan 2003; GRIN 2015; McGeoch *et al.* 2009; Retief & Herman 1997; Warwick *et al.* 2010). The herbarium survey resulted in 83 Brassicaceae species (see Appendix B) for the study area (Figure 4.1). Even though these specimens were collected subjectively in areas of interest (see Appendix C), the specimen data was assumed random and representative for the most abundant species. Not all the specimens in the herbaria were identified to species level. This is ascribed to the genetic variation that is known to occur in the Brassicaceae, ensuring considerable phenotypic plasticity (Bailey *et al.* 2006; Iqbal *et al.* 2008). Data analyses

took into account that a number of specimens were only identified to genus level (see Chapter 3.3).

The data included a total of 2296 specimens which 179 specimens were removed from the data set due to a lack of sufficient locality data, followed by removal of 683 specimens that occurred outside the study area. Cultivated species were removed from the remaining 1434 specimens, since these were collected from specialized, private and botanical gardens. 1374 specimen records remained for data analysis, comprising of 83 species.

4.2.3 Data standardization

The data used to determine the spatial distribution of the Brassicaceae is solely based on specimens retrieved from herbaria. This poses difficulty in that the data is subjective to collectors and warrants gaps in the distribution. Specimen collection data on its own is thus not sufficient to properly identify the spatial distribution of the Brassicaceae and was standardized as a means to logically fill the gaps in distribution data.

Kurzweg (2011) described a method for data standardization named the 'Centroid Grid profile'. This method of data standardization involved the transfer of species from three of the surrounding grids with the most similar vegetation units (Kurzweg 2011) (see Chapter 3.4). The Centroid Grid profile assumes that specific species will occur in similar vegetation units (Mucina & Rutherford 2006) and is used as a means to identify additional species that may be present in undersampled QDGC's (Kurzweg 2011).

4.2.4 Data analyses

PRIMER software (version 6) (PRIMER-E 2012) was used to perform ordinations with non-metric multidimensional scaling (NMDS) (Clarke & Gorley 2005). Non-parametric ordinations make no assumptions about probability distributions and the number of parameters increase as the data increases (Legendre & Anderson 1999). Data was pre-treatment standardized with square root transformations to reduce the contribution of outliers in respective samples of the total data set and remove negative eigenvalues (Legendre & Anderson 1999; Somerfield 2008). Bray-Curtis similarity index was applied to measure the resemblance between the sample abundances (Bray and Curtis 1957; Legendre & Anderson 1999; Somerfield 2008).

Initial non-parametric ordinations were performed to identify possible outlier QDGC's. Outliers were removed (see Chapter 3). This step was repeated three times. Dendrograms were constructed to classify grids into clusters with the most similar species composition. Similarity profile (SIMPROF) was used to indicate definite objective clusters (Clarke & Gorley 2005). A resemblance slice was drawn across the dendrogram to include no more than 15 and no less than seven clusters at a time (see Chapter 3.5.1). NMDS ordinations were performed. The clusters as identified by dendrograms, in conjunction with SIMPROF, were included in the NMDS by adding a *factor*; in this case, clusters of similar species composition to create a visual representation of the clustering of QDGC's.

Diagnostic species were identified for each of the clusters obtained. The amount of QDGC's per species in each cluster was used as a series value to determine the mean, standard deviation (σ) and upper quartile of the cluster. The standard deviation measures dispersion (similarity between species) by determining the average distance between the series values and the mean of the series as a whole (Arcidiacono 2010; UoL 2009). The closer the standard deviation is to 0, the more similar the species composition is within any specific cluster so that a high standard deviation indicates the presence of definite dominant species. The upper quartile is the value that indicates the uppermost 25% of values within the data series (Arcidiacono 2010; UoL 2009). Species were identified as being dominant when occurring in QDGC's equal to or above the upper quartile value.

The Paleontological Statistics (PAST) computer software package (Hammer *et al.* 2001) was used to determine similarity percentages (SIMPER) (Clarke 1993) and to conduct analyses of similarities (ANOSIM) of each of the non-standardized and standardized data sets (species, genera and tribes) (Table 4.1) (see Chapter 3.5.2). SIMPER is applied as a means to determine which species, genera or tribes (non-standardized and standardized) respectively were

responsible for the most dissimilarity (Clarke 1993), and ANOSIM as a means to determine whether significant differences are present in each of the six data sets (Table 4.1).

Table 4.1: Six different data sets were analysed in PRIMER and PAST.

Dataset	Hierarchy	Transformation
1	Species	Non-standardized
2	Species	Centroid Grid profile
3	Genera	Non-standardized
4	Genera	Centroid Grid profile
5	Tribe	Non-standardized
6	Tribe	Centroid Grid profile

4.2.5 Brassicaceae phytochoria

PRIMER was used to apply Bray-Curtis and SIMPROF to create dendrograms for each data set. These dendrograms identified clusters for each data set. The standardized Brassicaceae species was utilized for further analyses. Phytochoria were determined by comparing the presence of reproductively compatible relatives with bioregions and rainfall on the data set selected. The standardized species clusters containing reproductively compatible relatives were isolated and split into regions to define the distribution of the Brassicaceae across the study area.

4.3 Results

4.3.1 Specimen data

4.3.1.1 Non-standardized

A total of 1374 specimens were recorded for the QDGC of the study area, with the most hits belonging to tribe Lepidieae (381), genus *Lepidium* L. (302) and species *Heliophila rigidiuscula* Sond. (143). However, as the data was transformed to only reflect the presence/absence of species in any specific QDGC for data analysis, the hits were reduced from 1374 to 775. After transformation, Lepidieae and *Lepidium* occurred in the largest range of QDGC's, namely 218 and 164 respectively (Tables 4.2, 4.3). The species that occurred most frequently was *Lepidium africanum* (Burm.f.) DC. (52 grids), *Heliophila rigidiuscula* (44) and *Coronopus integrifolius* (DC.) Spreng. (41). Twenty species occurred in only one grid, of which 18 of these were only sampled once. The most specimens were collected in the provinces of the Free State (188) and the least in Limpopo (69), while the most species were recorded for the Gauteng and Free State (47), and the least in Limpopo (28) (Table 4.2).

Table 4.2: Proportion of species (n=83) per province (data standardized to include one hit per species per QDGC) across genera, expressed as a percentage. The dominant genus in each province is indicated in bold.

Genera	Indigenous (I), Alien (A)	Provinces						Total study area
		Gauteng	Limpopo	Mpumalanga	North-West	Free State	Northern Cape	
<i>Alyssum</i> L.	A (1)	0.351	0	0	0	0	0	0.351
<i>Armoracia</i> P. Gaertn.	A (1)	0.351	0	0	0	0	0	0.351
<i>Brassica</i> L.	A (5)	0.702	1.404	1.053	1.754	1.404	0.702	7.018
<i>Camelina</i> Crantz	A (1)	0.351	0	0	0.351	0	0	0.702
<i>Capsella</i> Medik.	A (1)	0.351	0	0.351	0.351	0.351	0.351	1.754
<i>Cardamine</i> L.	I (1) / A (3)	0.702	0.351	1.053	0.351	1.053	0	3.509
<i>Coronopus</i> Zinn	A (2)	0.702	0.351	0.702	0.702	0.702	1.404	4.561
<i>Descurainia</i> Webb&Berthel.	A (1)	0.351	0	0.351	0	0	0	0.702
<i>Diploaxis</i> DC.	A (2)	0.351	0.351	1.053	1.053	1.053	0.351	4.211
<i>Eruca</i> Mill.	A (1)	0.351	0	0	0.351	0.351	0	1.053
<i>Erucastrum</i> (DC.) C.Presl	I (3)	0.702	0.702	0.702	1.754	1.404	1.053	6.316
<i>Erysimum</i> L.	A (1)	0.351	0	0	0	0	0	0.351
<i>Heliophila</i> L.	I (20)	2.456	2.456	2.105	2.456	0.351	0.3513	16.14
<i>Lepidium</i> L.	I (12) / A (2)	0.351	2.456	2.807	2.105	3.158	3.158	17.193
<i>Lobularia</i> Desv.	A (1)	0.351	0	0	0	0	0	0.351
<i>Matthiola</i> R.Br.	I (1) / A (1)	0.351	0.351	0	1.053	0.351	1.404	3.509
<i>Nasturtium</i> R.Br.	A (1)	0.351	0.351	0.351	0.351	0.351	0.351	2.105
<i>Raphanus</i> L.	A (2)	1.053	0.702	0.702	0.702	0.702	0.351	4.121
<i>Rapistrum</i> Crantz	A (1)	0.351	0.351	0	0.351	0.351	0.351	1.754
<i>Rorippa</i> Scop.	I (4)	1.053	1.053	1.053	1.053	1.053	0.702	5.965
<i>Sinapis</i> L.	A (2)	0.702	0.702	0.351	0.702	0.351	0	2.807
<i>Sisymbrium</i> L.	I (5) / A (3)	2.807	0.351	2.456	2.807	0.351	2.105	14.035
<i>Turritis</i> L.	A (1)	0	0.351	0.351	0	0.351	0	1.053
<i>n</i> (genera) = 23	<i>n</i> (species) = 83	17.893	11.579	15.439	18.246	20.0	16.842	100

Table 4.3: Proportion of species (n=83) per province (data standardized to include one hit per species per QDGC) across tribes, expressed as a percentage. The dominant tribe in each province is indicated in bold.

Tribe	Indigenous (I), Alien (A)	Provinces						Total study area
		Gauteng	Limpopo	Mpuma- langa	North- West	Free State	Northern Cape	
Alysseae DC.	A (2)	0.702	0	0	0	0	0	0.702
Anchonieae DC.	I (2) / A (1)	0.351	0.351	0	1.053	0.351	1.404	3.509
Brassicaceae DC.	I (4) / A (12)	4.212	4.212	3.861	6.667	5.616	2.808	27.28
Camelineae DC.	A (3)	0.702	0.351	0.702	0.702	0.702	0.351	3.509
Cardamineae Dumort.	I (5) / A (5)	2.457	1.755	2.457	1.755	2.457	1.053	11.93
Descurainieae Al-Shehbaz, Beilstein & E.A. Kellogg	A (1)	0.351	0	0.351	0	0	0	0.702
Erysimeae Dumort.	A (1)	0.351	0	0	0	0	0	0.351
Heliophileae DC.	I (20)	2.456	2.456	2.105	2.456	0.351	0.3513	16.14
Lepidieae DC.	I (13) / A (5)	1.053	2.807	3.509	2.807	3.86	4.562	21.754
Sisymbrieae DC.	I (6) / A (3)	2.807	0.351	2.456	2.807	0.351	2.105	14.035
<i>n</i> (tribes) = 10	<i>n</i> (species) = 83	17.893	11.579	15.439	18.246	20.0	16.842	100

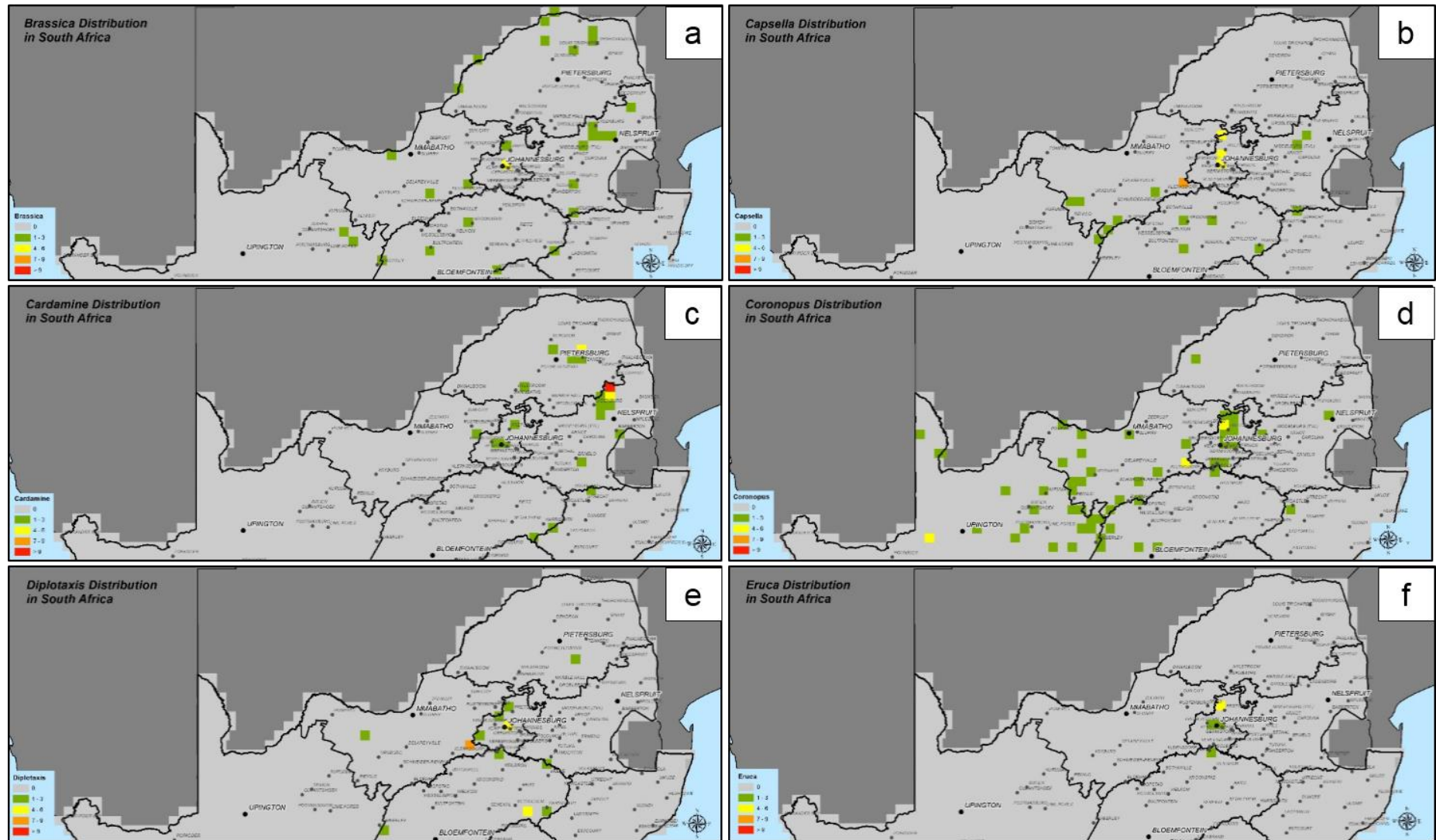


Figure 4.3: Non-standardized distribution of individuals of the most abundant Brassicaceae genera: *Brassica* (a), *Capsella* (b), *Cardamine* (c), *Coronopus* (d), *Diplotaxis* (e), and *Eruca* (f).

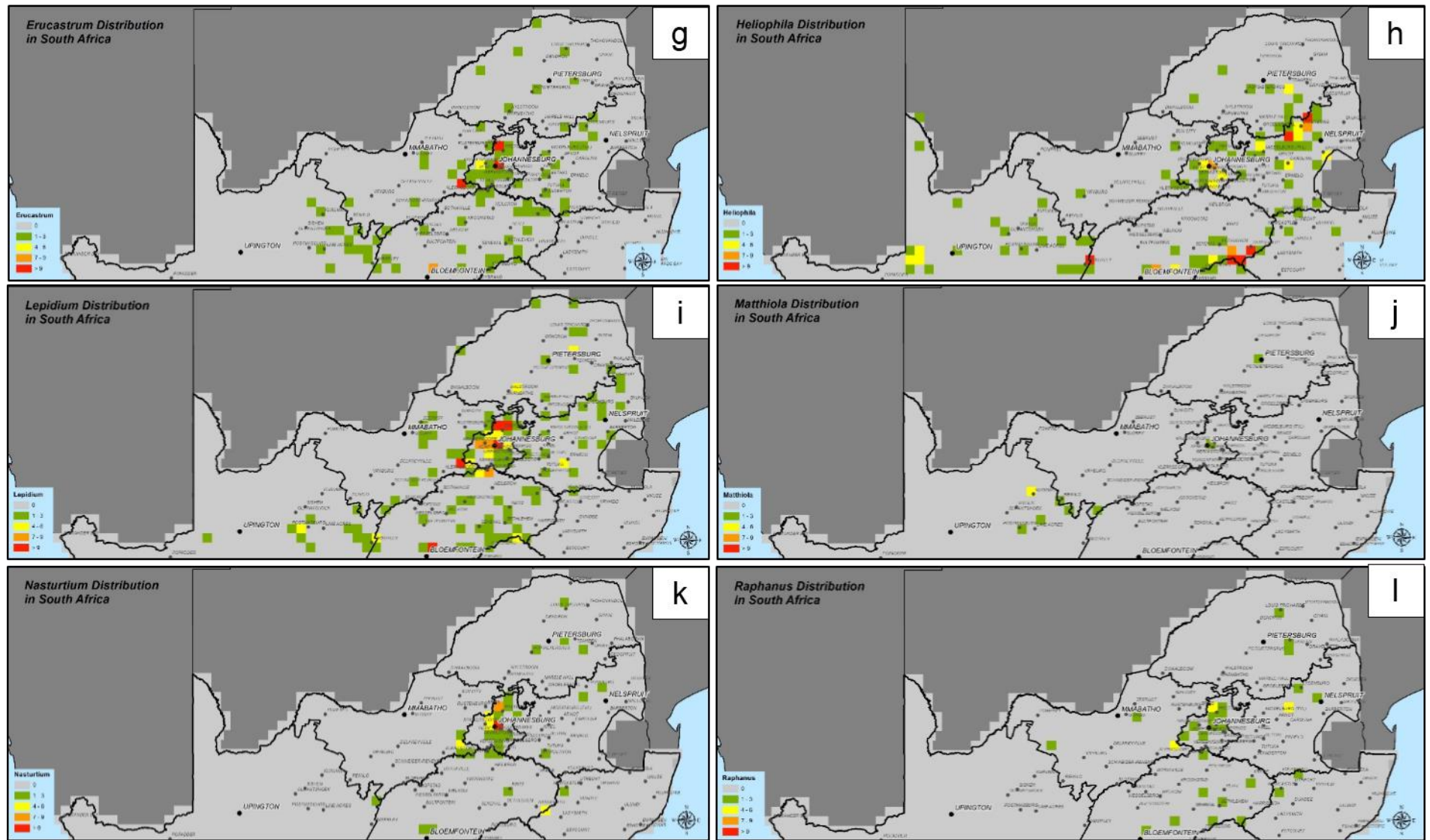


Figure 4.3 (continued): *Erucastrum* (g), *Heliophila* (h), *Lepidium* (i), *Matthiola* (j), *Nasturtium* (k), and *Raphanus* (l).

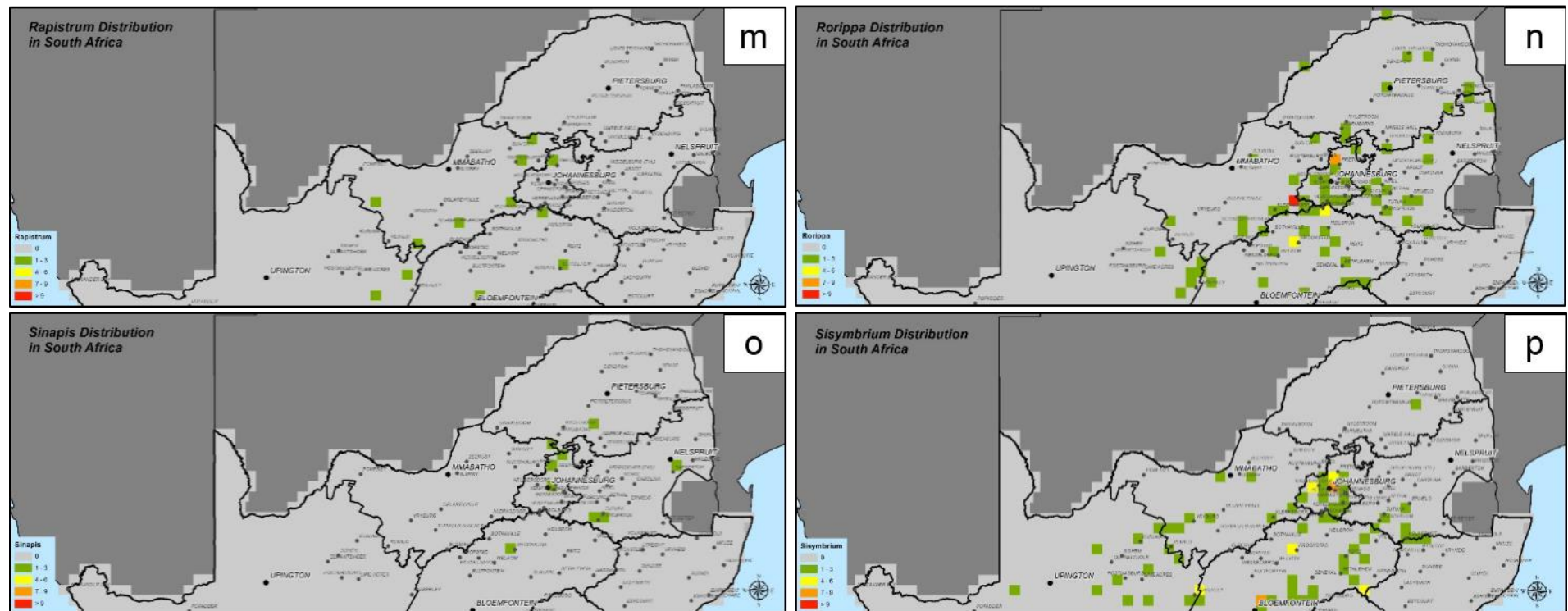


Figure 4.3 (concluded): *Rapistrum* (m), *Rorippa* (n), *Sinapis* (o), and *Sisymbrium* (p).

4.3.1.2 Standardized data

Standardizing the data by Centroid Grid profile increased the presence/absence hits per species per QDGC in the study area from 775 to 2726. The maximum number of species in the non-standardized data set was 30 for a single QDGC (2628AA, central Gauteng), followed by 21 (2528CA, north Gauteng) and 19 (2627CA, eastern North-West). These numbers increased significantly when the Centroid Grid profile was applied to the data (Figure 4.4). The maximum numbers of species in a single QDGC increased to 38 in both 2627BB and 2628AA (central Gauteng), followed by 34 (2528CC, central Gauteng) and 33 (2528CD, central Gauteng).

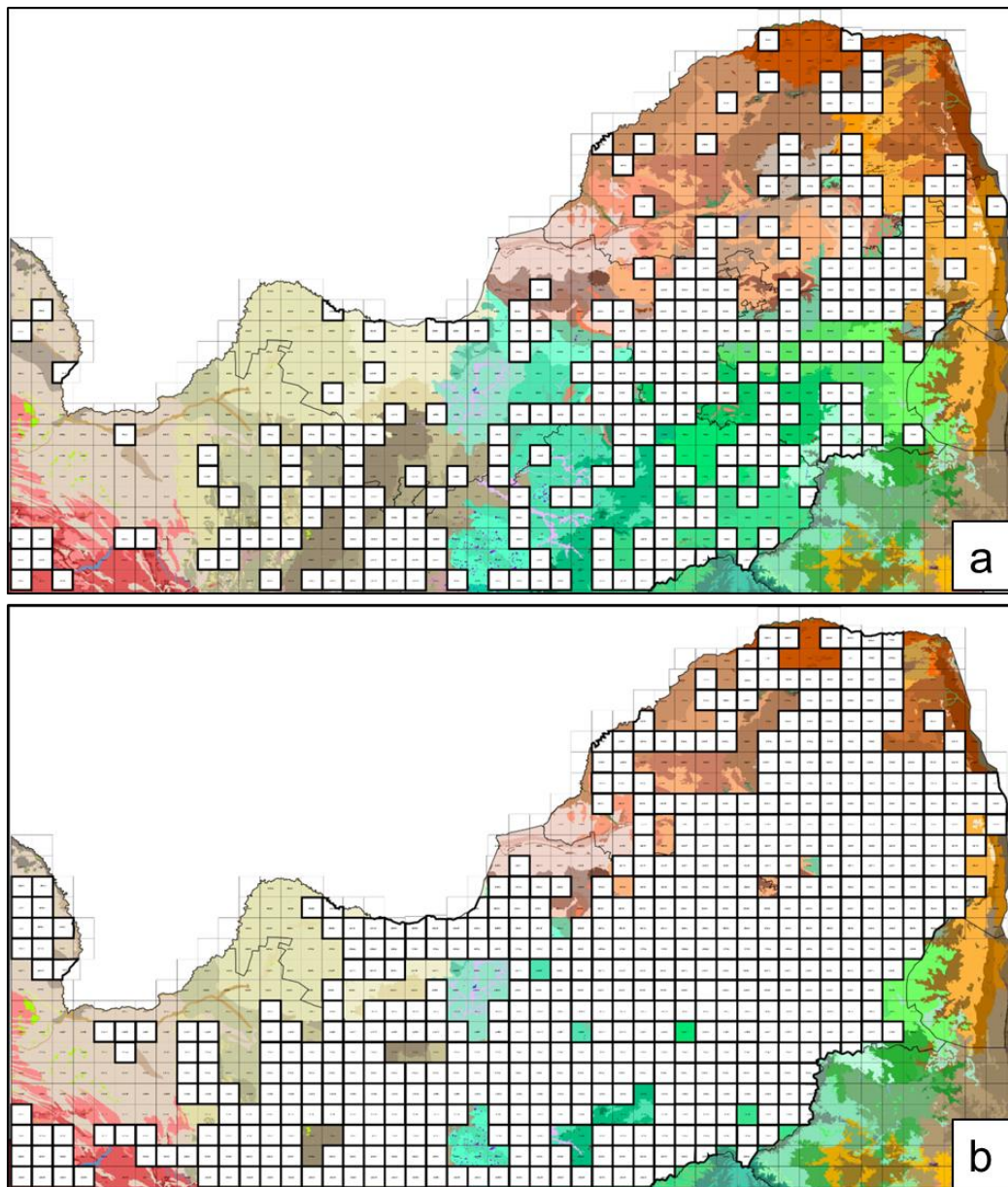


Figure 4.4: Brassicaceae distribution pattern generated from non-standardized (a) to Centroid Grid profile (b), data across the study area based on presence/absence data. White cells indicate the presence of at least one Brassicaceae for a QDGC.

The non-standardized distribution of the Brassicaceae (Figure 4.4a) was increased from 264 to 559 QDGC's with the Centroid Grid profile (Kurzweg 2011) (Figure 4.4b). The increase in QDGC's between non-standardized specimen data and Centroid Grid profile standardization is indicated in Figure 4.5.

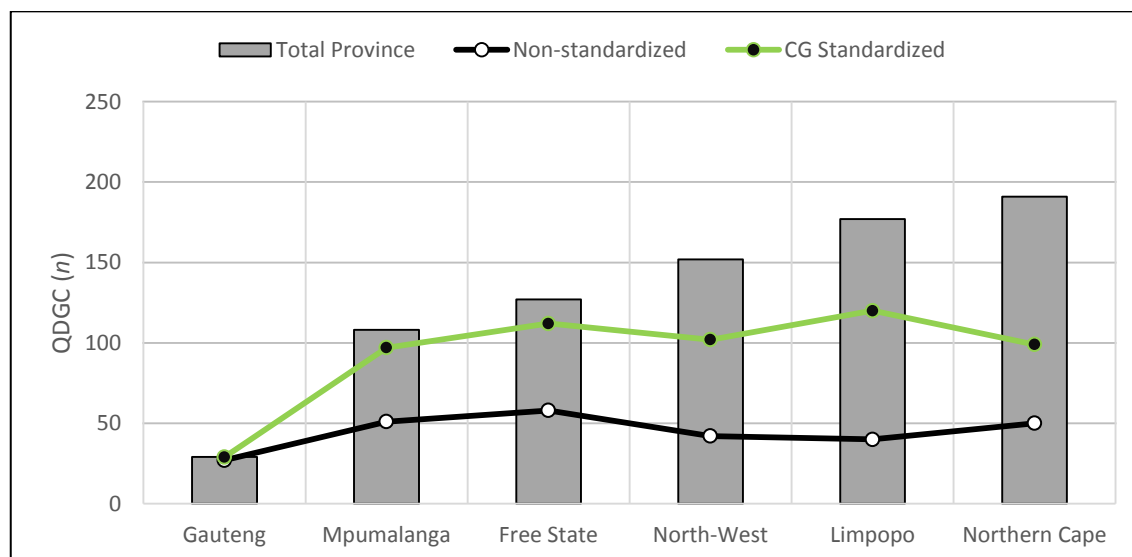


Figure 4.5: QDGC's of both non-standardized and Centroid Grid profile (CG) standardization.

The species data per province increased after application of the Centroid Grid profile (Figure 4.6, 4.7) with large increases considered a correction for poor collection effort. Application of the Centroid Grid profile further increases the distribution of the Brassicaceae in areas with low population densities that may have been undercollected (Figure 4.7).

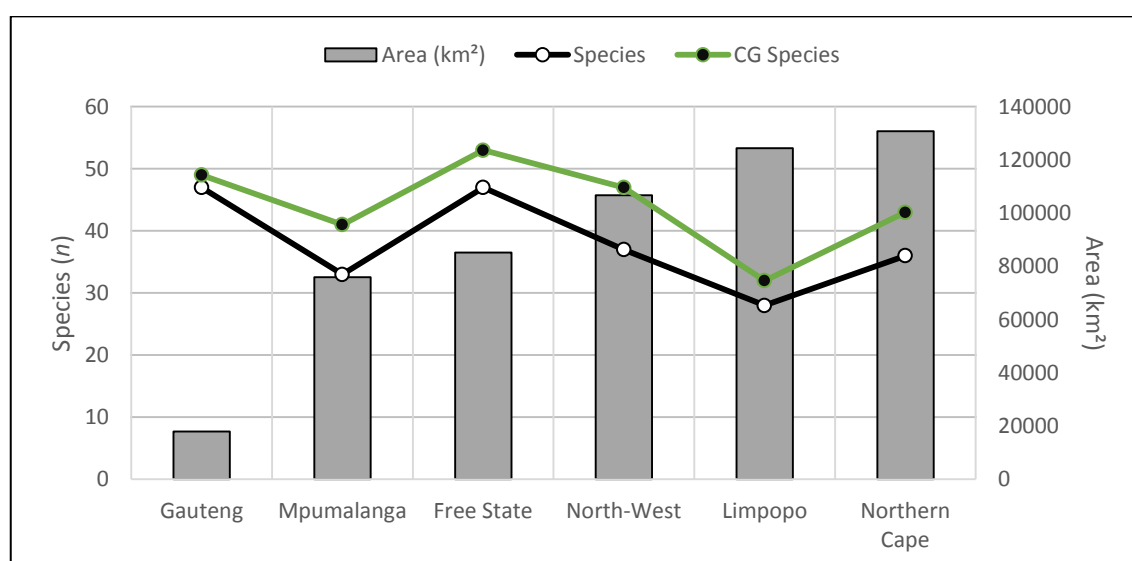


Figure 4.6: Non-standardized and Centroid Grid profile (CG) species richness per province.

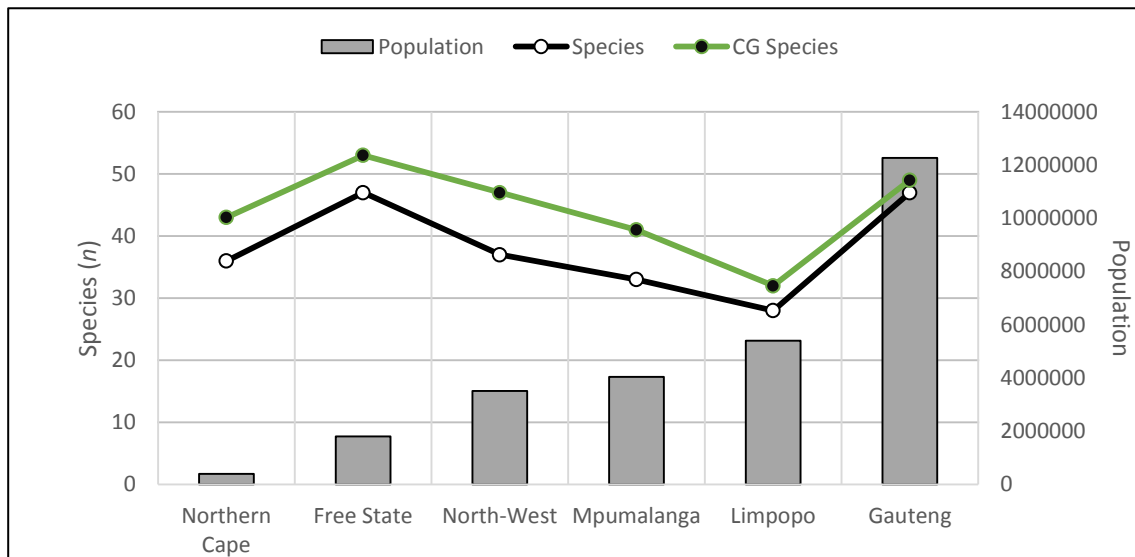


Figure 4.7: Non-standardized and Centroid Grid profile standardized species compared to population density (SSA 2014).

4.3.2 Species assemblages

4.3.2.1 Non-standardized

The non-standardized species QDGC's were subjected to NMDS in PRIMER. Multiple outliers were present and removed to normalize the data for improved analysis (see Chapter 3: 3.5.1). 2230AC, 2331CD, 2430CC, 2431BD, 2525CC, 2528AA, 2630CC, 2721AB and 2823CC were removed first, followed by 2230CD, 2429CA, 2531CA, 2626CC, 2629CC, 2820CC, 2820DC, 2824CB and 2826AC, and 2723CB, 2727AB, 2820AC, 2820AD, 2820CA, 2821AD and 2824CC were removed last (25 of 799 = 3.13%) (Figure 4.8a). After outliers were removed, the final NMDS graph indicated no clusters (Figure 4.8b).

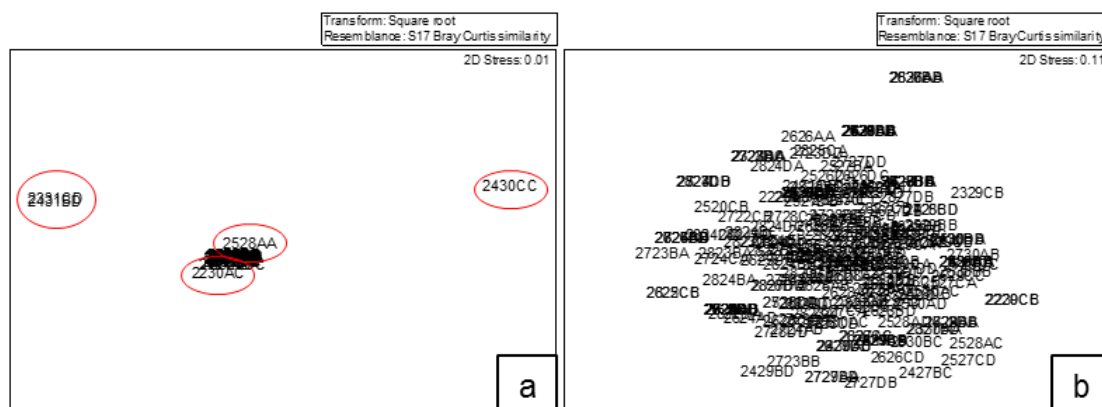


Figure 4.8: Initial non-standardized species NMDS graph before outliers were removed (a) and after (b). Red circles (a) indicate the first set of subjectively chosen outliers.

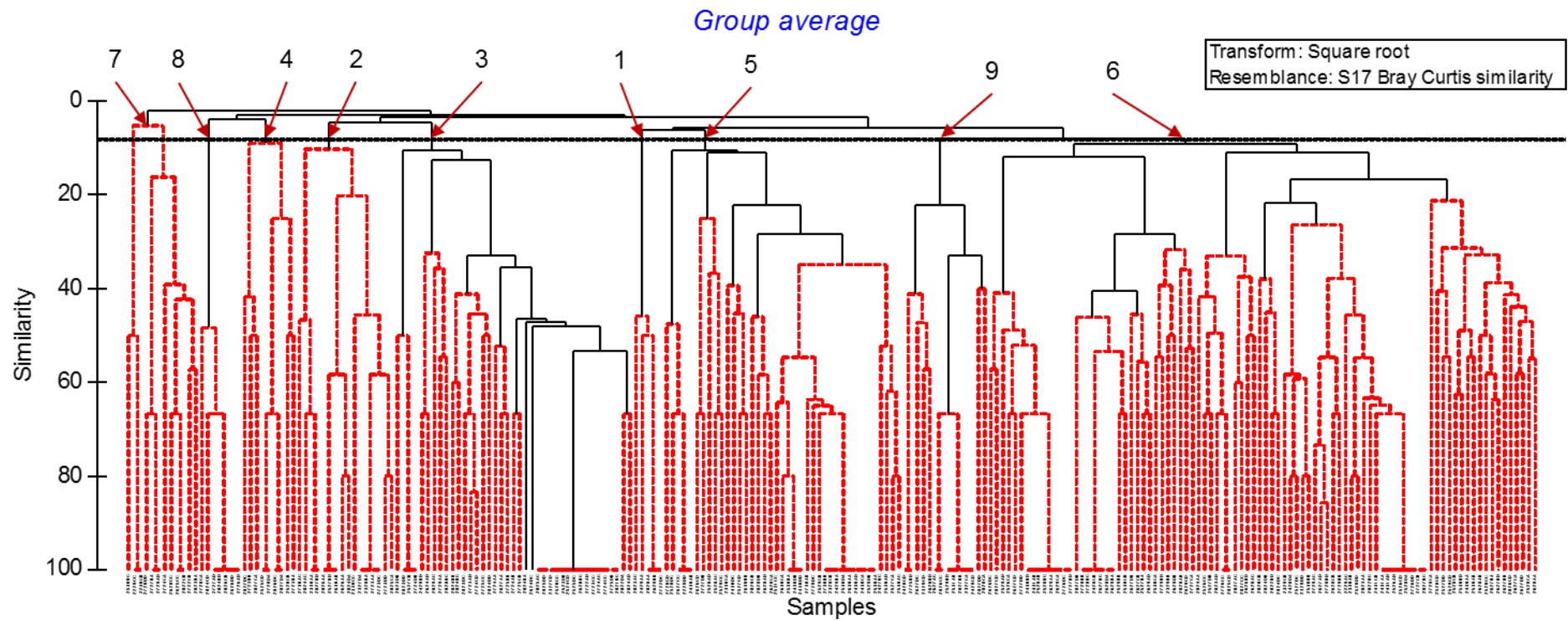


Figure 4.9: Dendrogram for non-standardized species data, including SIMPROF test (dotted red lines). Each sample is a QDGC. Resemblance slice (horizontal black line) was set at 8%. Clusters are indicated by numbers.

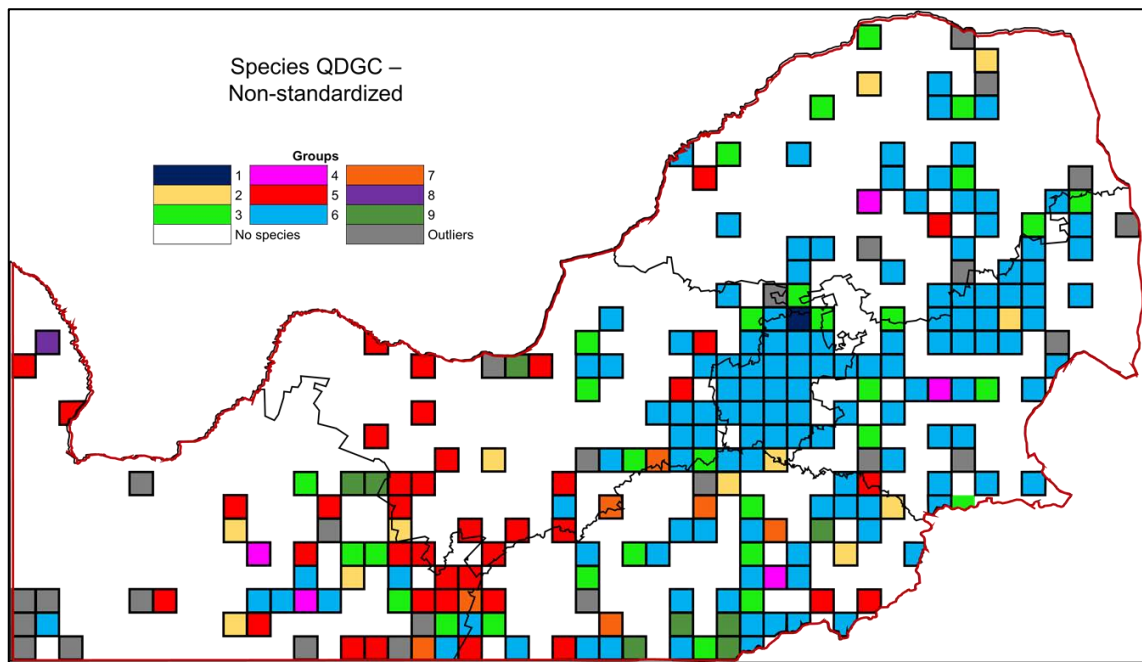


Figure 4.11: Distribution of non-standardized species data across the study area, indicating clusters with similar species composition as determined by Bray-Curtis and SIMPROF. Groups = clusters.

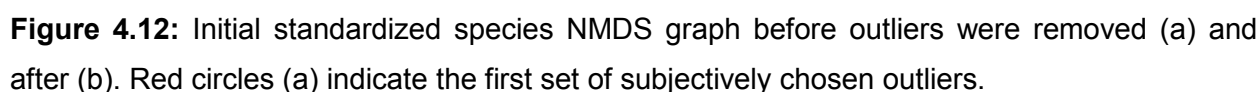
The diagnostic species were identified for each of the non-standardized species clusters and is indicated in Table 4.4. Several clusters contained diagnostic species that occurred regularly throughout the QDGC's of each cluster and was identified as dominant when occurring in QDGC's equal to or above the upper quartile value (Table 4.4).

Cluster one had four species that each occurred once in a QDGC (Table 4.4). The mean is therefore one and the standard deviation consequently zero, indicating that there is no difference in number of species between different QDGC's within the cluster. Cluster 6, the largest cluster as determined by dendrogram, has the highest standard deviation (22.57). The high standard deviation further contributes to clarification of the low upper quartile (16) and indicates that 75% of each species (within the 973 total individuals) occur 16 times in the cluster respectively. A high standard deviation and low upper quartile number thus indicates that the QDGC's contain species occurring in a variety of frequencies. When considering the standard deviation and upper quartiles of each cluster, and keeping in mind the total specimens of that specific cluster, the cogency of every cluster within each data set may be estimated.

Table 4.4: Species clusters (non-standardized data), number of grids, specimens and the dominant species present. Coloured squares coincide with clusters as indicated in Figures 4.9, 4.11. Species in bold occur throughout the most QDGC's within that cluster.

NON-STANDARDIZED SPECIES CLUSTERS						
Cluster number	Total number of grids	Mean	Standard deviation	Upper quartile (75%)	Number of individuals	Diagnostic species
1	1	1	0	1	4	<i>Coronopus didymus</i> <i>Lepidium virginicum</i> <i>Nasturtium officinale</i> <i>Sinapis arvensis</i>
2	12	3.8	3.27	4	27	<i>Brassica rapa</i> <i>Sisymbrium burchellii</i> var. <i>burchellii</i> <i>Sisymbrium thellungi</i>
3	31	3.29	4.15	3	28	<i>Erucastum griquense</i> <i>Lepidium africanum</i> <i>Lepidium schinzii</i> <i>Lepidium species</i> <i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>
4	5	1.8	1.78	1	9	<i>Erucastum griquense</i> <i>Heliophila carnos</i> <i>Heliophila suavissima</i> <i>Matthiola torulosa</i> <i>Nasturtium officinale</i>
5	39	5.96	8.61	6.5	163	<i>Coronopus integrifolius</i> <i>Diplotaxis muralis</i> <i>Erucastum griquense</i> <i>Erucastum strigosum</i> <i>Heliophila carnos</i> <i>Heliophila rigidiuscula</i> <i>Rapistrum rugosum</i>
6	127	13.73	22.57	16	973	<i>Capsella bursa-pastoris</i> <i>Cardamine africana</i> <i>Coronopus integrifolius</i> <i>Erucastum austroafricanum</i> <i>Erucastum strigosum</i> <i>Heliophila carnos</i> <i>Heliophila rigidiuscula</i> <i>Lepidium africanum</i> <i>Lepidium bonariense</i> <i>Lepidium transvaalense</i> <i>Lepidium virginicum</i> <i>Nasturtium officinale</i> <i>Raphanus raphanistrum</i> <i>Rorippa fluviatilis</i> var. <i>fluviatilis</i> <i>Rorippa nudiuscula</i> <i>Sisymbrium orientale</i> <i>Sisymbrium thellungii</i> <i>Sisymbrium turczaninowii</i>
7	7	2.86	2.98	1.5	17	<i>Rorippa fluviatilis</i> var. <i>caledonica</i>
8	1	1	0	1	2	<i>Heliophila deserticola</i> <i>Heliophila suavissima</i>
9	8	2.43	1.99	3.5	17	<i>Heliophila suavissima</i> <i>Sisymbrium turczaninowii</i>

The standardized QDGC's were subjected to NMDS in PRIMER (Figure 4.12).



As a means to determine clusters, a Bray-Curtis similarity dendrogram was derived for standardized species data to indicate clusters of QDGC's with similar species composition. The dendrogram for Centroid Grid profile standardized species had multiple divisions from a low resemblance, so that a low resemblance, albeit higher than with the non-standardized species data, was chosen for clustering (10%, Figure 4.13). Similarity profile (SIMPROF) indicated definite objective clusters. The resemblance slice was applied to merge some SIMPROF clusters at the first definite junction (Figure 4.13). Clusters indicated by dendrogram and SIMPROF were inserted into the NMDS using Factors to create a final NMDS that contains twelve clusters (Figure 4.14).

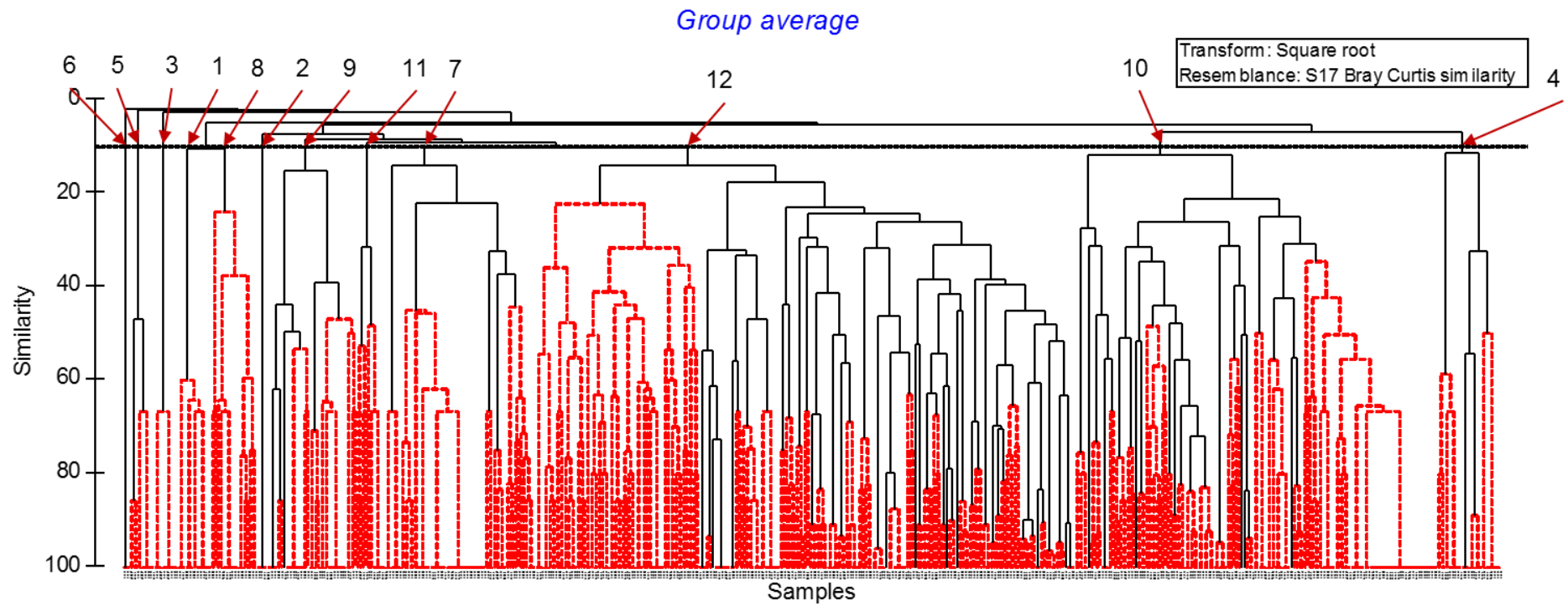


Figure 4.13: Dendrogram for standardized (CG) species, indicating SIMPROF test (dotted red lines). Each sample is a QDGC. Resemblance slice (horizontal black line) was set at 10%. Clusters are indicated by numbers.

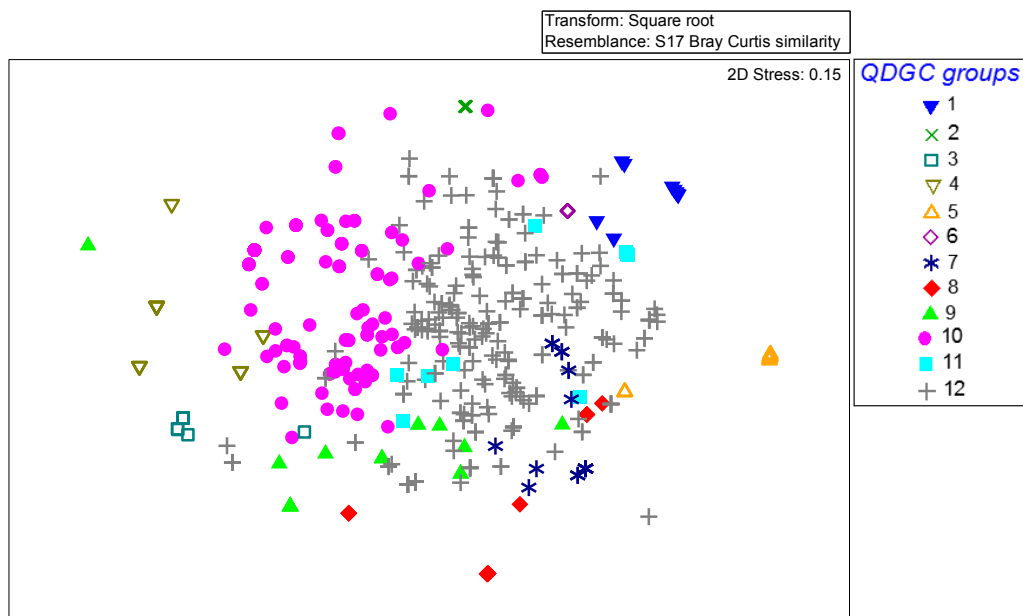


Figure 4.14: NMDS CG standardized species, indicating clusters with similar species compositions as determined by Bray-Curtis similarity and SIMPROF. Groups = clusters.

These standardized clusters of species assemblages were indicated on a map of the study area by colouring each QDGC accordingly (Figure 4.15). The outliers were indicated, since it is possible for these QDGC's to form their own cluster.

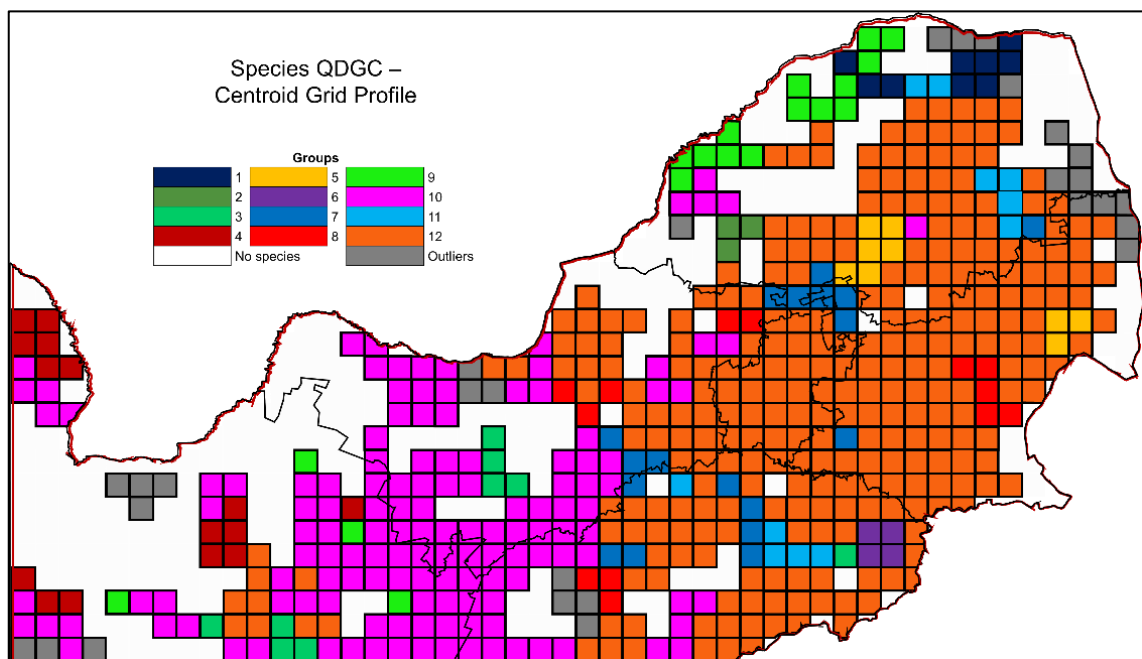


Figure 4.15: Distribution of species clusters based on the Centroid Grid profile across the study area. Clusters with similar species composition was determined by Bray-Curtis similarity and SIMPROF. Groups = clusters.

Standardized assemblages of species were overlapped with bioregions (Mucina & Rutherford 2006) (Figure 4.16), seasonal rainfall (SAWS 2015) (Figure 4.17a) and mean rainfall over 50 years (Lynch 2004) (Figure 4.17b) maps as a means of identifying possible factors influencing the distribution of the Brassicaceae.

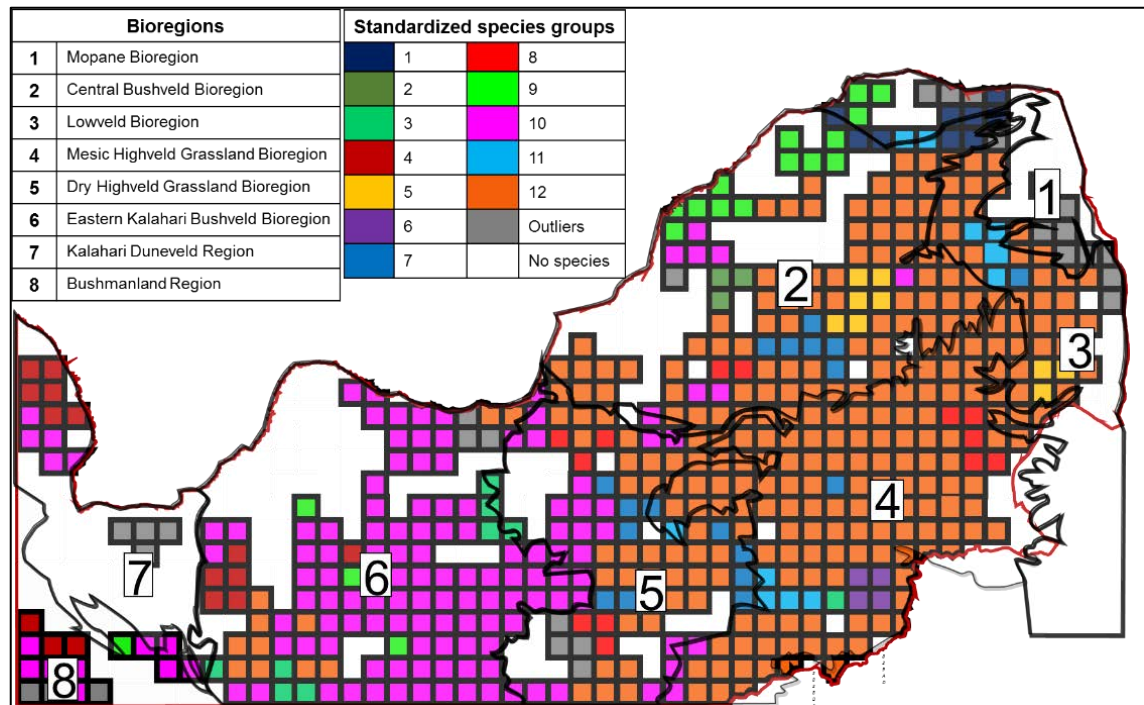


Figure 4.16: Distribution of standardized species clusters, as determined by Bray-Curtis and SIMPROF; indicated in coloured areas. Species clusters are overlapped with bioregions (Mucina & Rutherford 2006), outlined in black and numbered for identification. Groups = clusters.

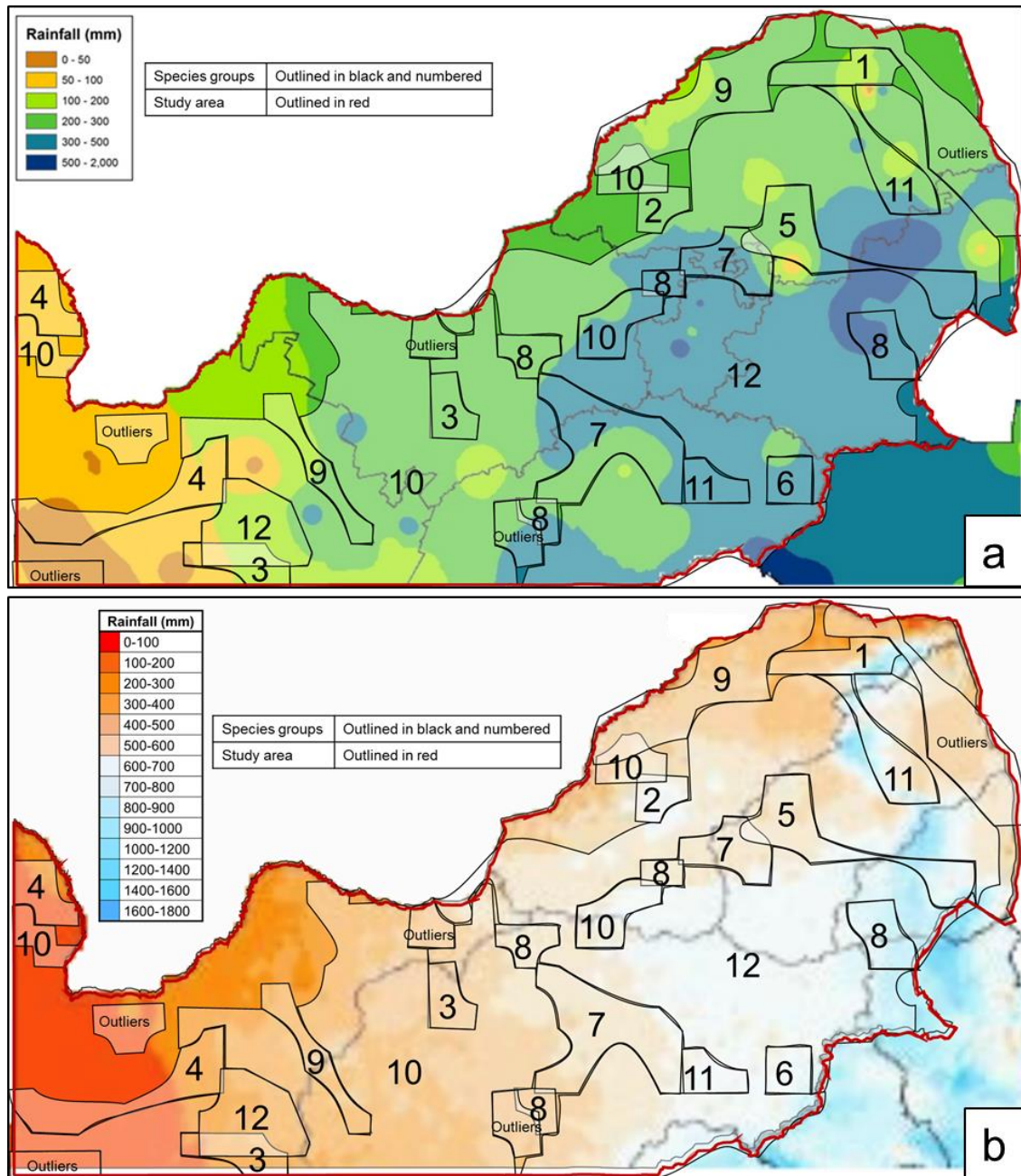


Figure 4.17: Seasonal rainfall (SAWS 2015) (a) and mean rainfall between 1950 and 1999 (Lynch 2004) (b) overlapped with the distribution of standardized species clusters (as determined by Bray-Curtis and SIMPROF). Species clusters are outlined in black and numbered. Groups = Clusters.

The dominant species were identified for each of the standardized species clusters and is indicated in Table 4.5. Several clusters contained diagnostic species that occurred regularly throughout the QDGC's of each cluster and was identified as dominant when occurring in QDGC's equal to or above the upper quartile value (Table 4.5).

Table 4.5: Species clusters (standardized data), number of grids, specimens and the dominant species present. Coloured squares coincide with clusters as indicated in figures 4.13, 4.15. Species in bold occur throughout the most QDGC's within that cluster.

STANDARDIZED SPECIES CLUSTERS						
Cluster number	Total number of grids	Mean	Standard deviation	Upper quartile (75%)	Number of individuals	Dominant species
1	9	4	3.16	5.75	16	<i>Brassica rapa</i>
2	3	3	–	3	3	<i>Heliophila</i> species
3	9	3.5	3.79	4.5	14	<i>Sisymbrium burchellii</i> var. <i>burchellii</i>
4	15	6.43	3.95	8.5	45	<i>Heliophila deserticola</i> <i>Heliophila suavissima</i>
5	9	6	4.24	7.5	12	<i>Sinapis arvensis</i>
6	4	4	0	4	8	<i>Lepidium transvaalense</i> <i>Raphanus raphanistrum</i>
7	18	4.44	4.93	5	40	<i>Capsella bursa-pastoris</i> <i>Rorippa fluviatilis</i> var. <i>fluviatilis</i> <i>Sisymbrium</i> species
8	13	5	5.42	5.5	20	<i>Lepidium</i> species
9	18	4.88	4.64	5.75	39	<i>Brassica elongata</i> <i>Erucastrum griquense</i>
10	138	13.93	18.14	19	627	<i>Capsella bursa-pastoris</i> <i>Coronopus integrifolius</i> <i>Erucastrum griquense</i> <i>Erucastrum strigosum</i> <i>Heliophila minima</i> <i>Heliophila suavissima</i> <i>Lepidium africanum</i> <i>Matthiola torulosa</i> <i>Rapistrum rugosum</i> <i>Rorippa fluviatilis</i> var. <i>caledonica</i> <i>Sisymbrium burchellii</i> var. <i>burchellii</i> <i>Sisymbrium capense</i>
11	11	2.75	2.83	3.25	33	<i>Lepidium trifurcum</i> <i>Nasturtium officinale</i> <i>Sisymbrium turczaninowii</i>
12	277	26.13	29.78	39	1802	<i>Brassica rapa</i> <i>Cardamine africana</i> <i>Diplotaxis muralis</i> <i>Erucastrum austroafricanum</i> <i>Erucastrum strigosum</i> <i>Heliophila carnosa</i> <i>Heliophila rigidiuscula</i> <i>Heliophila</i> species <i>Lepidium africanum</i> <i>Lepidium bonariense</i> <i>Lepidium schinzii</i> <i>Lepidium transvaalense</i> <i>Lepidium virginicum</i> <i>Nasturtium officinale</i> <i>Raphanus raphanistrum</i> <i>Rorippa fluviatilis</i> var. <i>fluviatilis</i> <i>Rorippa nudiuscula</i> <i>Sisymbrium thellungii</i> <i>Sisymbrium turczaninowii</i>

Cluster 10 of the standardized species distribution clusters as identified with the use of dendrograms is mostly present within the Eastern Kalahari Bushveld bioregion (Figure 4.16). Cluster four and two of the outlier clusters only occurs where the rainfall is between 0 and 100 mm (Figure 4.17). Clusters six, eight and 12 is distributed across the study area where the season rainfall is higher than 200 mm (Figure 4.17). These three clusters each contain a *Lepidium* species as the dominant species.

4.3.3 Genus assemblages

4.3.3.1 Non-standardized

The non-standardized genera QDGC's were subjected to NMDS in PRIMER. Multiple outliers were present and removed to normalize the data for improved analysis (see Chapter 3: 3.5.1). 2429CA, 2528AA, 2531CA and 2629CC were removed first (Figure 4.18a), 2429BD, 2523DD, 2527CD and 2724DB second, and 2527CB and 2727DB were removed thirdly (10 of 799 = 1.25%). After outliers were removed, the final NMDS graph indicated no clusters (Figure 4.18b).

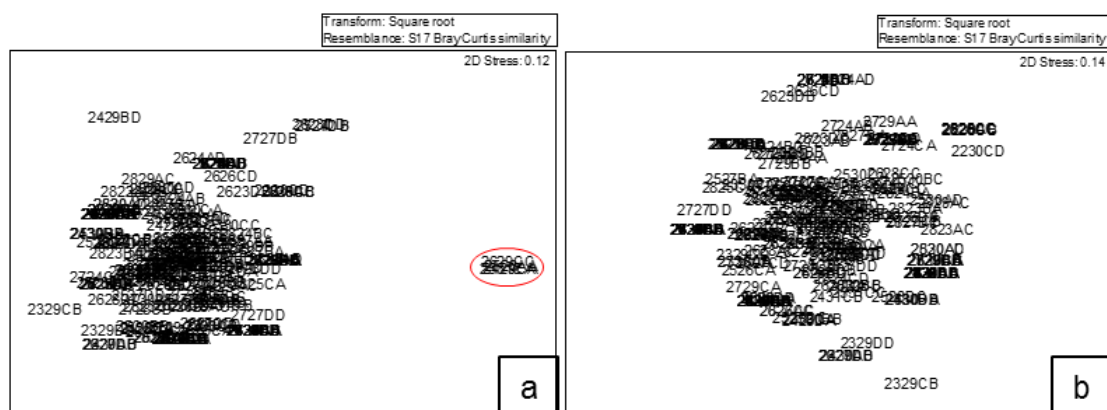


Figure 4.18: Initial non-standardized genera NMDS graph before outliers were removed (a) and after (b). Red circles (a) indicate the first set of subjectively chosen outliers.

As a means to determine clusters, a Bray-Curtis similarity dendrogram was derived for non-standardized genus data to indicate clusters of QDGC's with similar species composition. The dendrogram analysis for non-standardized genera had multiple divisions after a 45% resemblance, so that a resemblance was chosen (30%, Figure 4.19) where the number of clusters could be indicated on a study area graph with ease. Similarity profile (SIMPROF) indicated definite objective clusters. The resemblance slice was applied to merge some SIMPROF clusters at the first definite junction (Figure 4.19). Clusters indicated by dendrogram and SIMPROF were inserted into the NMDS using Factors to create a final NMDS that contains nine clusters (Figure 4.20). The remaining QDGC clusters were inserted into PRIMER, and the resulting NMDS contained seven clusters (Figure 4.20).

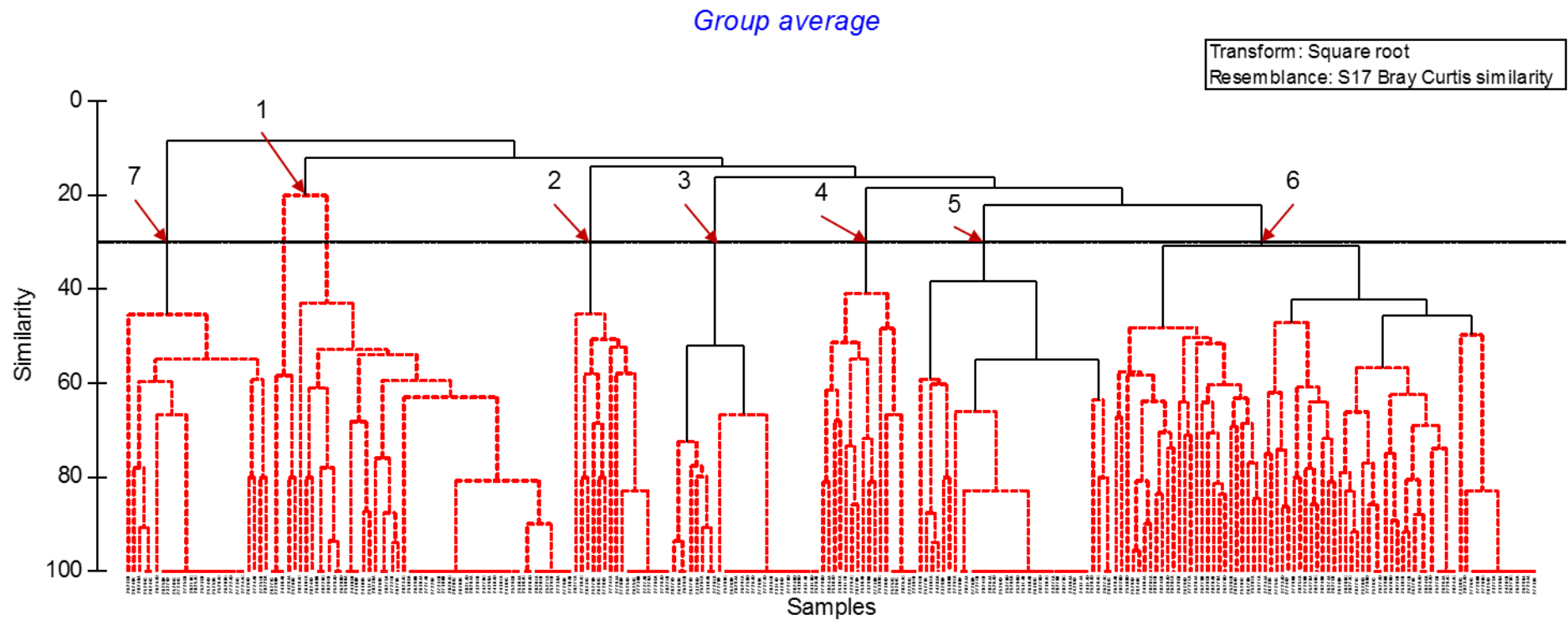


Figure 4.19: Dendrogram for non-standardized genera, indicating SIMPROF test (dotted red lines). Each sample is a QDGC. Resemblance slice (horizontal black line) was set at 30%. Clusters are indicated by numbers.

The dominant species and genera for each of the non-standardized genera clusters was identified and is indicated in Table 4.6. Several clusters contained diagnostic species and genera that occurred regularly throughout the QDGC's of each cluster and was identified as dominant when occurring in QDGC's equal to or above the upper quartile value (Table 4.6).

Table 4.6: Genera clusters (non-standardized data) as determined by dendrogram, indicating number of QDGC's, specimens and the dominant species and genera present. Coloured squares coincide with clusters as indicated in figures 4.19, 4.21. Species and genera in bold occur throughout the most QDGC's within that cluster.

NON-STANDARDIZED GENERA CLUSTERS						
Cluster number	Total number of grids	Mean	Standard deviation	Upper quartile (75%)	Number of individuals	Dominant species / genus
SPECIES						
1	53	6.19	13.63	4.5	192	<i>Cardamine africana</i> <i>Heliophila carnosa</i> <i>Heliophila deserticola</i> <i>Heliophila rigidiuscula</i> <i>Heliophila</i> species <i>Heliophila suavissima</i> <i>Heliophila trifurca</i> <i>Nasturtium officinale</i>
2	17	2.33	1.78	3	42	<i>Diplotaxis muralis</i> <i>Nasturtium officinale</i> <i>Sisymbrium burchellii</i> var. <i>burchellii</i> <i>Sisymbrium</i> species <i>Sisymbrium thellungii</i> <i>Sisymbrium turczaninowii</i>
3	26	3.73	3.83	4	56	<i>Lepidium africanum</i> <i>Rorippa fluviatilis</i> var. <i>caledonica</i> <i>Rorippa fluviatilis</i> var. <i>fluviatilis</i> <i>Rorippa nudiuscula</i>
4	17	3.29	3.49	4	69	<i>Brassica juncea</i> <i>Brassica rapa</i> <i>Erucastrum austroafricanum</i> <i>Heliophila rigidiuscula</i> <i>Lepidium africanum</i> <i>Raphanus raphanistrum</i>
5	34	4.47	5.08	6.5	67	<i>Cardamine africana</i> <i>Lepidium africanum</i> <i>Lepidium bonariense</i> <i>Nasturtium officinale</i>
6	74	11.71	15.98	16	808	<i>Capsella bursa-pastoris</i> <i>Coronopus integrifolius</i> <i>Diplotaxis muralis</i> <i>Erucastrum austroafricanum</i> <i>Erucastrum griquense</i> <i>Erucastrum strigosum</i> <i>Heliophila carnosa</i> <i>Heliophila rigidiuscula</i> <i>Lepidium africanum</i> <i>Lepidium bonariense</i> <i>Lepidium transvaalense</i> <i>Nasturtium officinale</i> <i>Raphanus raphanistrum</i>

							<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i> <i>Rorippa nudiuscula</i> <i>Sisymbrium orientale</i> <i>Sisymbrium thellungii</i> <i>Sisymbrium turczaninowii</i>
	7	25	3.4	7.12	2	51	<i>Capsella bursa-pastoris</i> <i>Coronopus didymus</i> <i>Coronopus integrifolius</i> <i>Diploaxis muralis</i> <i>Heliophila bonariense</i> <i>Heliophila suavissima</i> <i>Rapistrum rugosum</i>
GENUS							
	1	53	19.2	38.63	9.75	192	<i>Cardamine</i> <i>Heliophila</i> <i>Lepidium</i>
	2	17	6	6.66	7	42	<i>Lepidium</i> <i>Sisymbrium</i>
	3	26	9.33	12.31	12.5	56	<i>Lepidium</i> <i>Rorippa</i>
	4	17	7.56	6.67	8	69	<i>Brassica</i> <i>Erucastrium</i> <i>Heliophila</i>
	5	34	9.57	15.7	9.5	67	<i>Cardamine</i> <i>Lepidium</i>
	6	74	35.13	50.97	43.5	808	<i>Erucastrium</i> <i>Heliophila</i> <i>Lepidium</i> <i>Nasturtium</i> <i>Rorippa</i> <i>Sisymbrium</i>
	7	25	6.38	10.86	3.75	51	<i>Coronopus</i> <i>Heliophila</i>

4.3.3.2 Standardized

The standardized genera QDGC's were subjected to NMDS in PRIMER. Multiple outliers were present and removed to normalize the data for improved analysis (see Chapter 3: 3.5.1). 2329AD, 2428DD, 2429AC, 2429AD, 2429BC, 2429CA, 2527CB, 2531AC, 2531CA and 2725CA were identified as outliers and removed (10 of 2726 = 0.37%) (Figure 4.22a). After outliers were removed, the final NMDS graph indicated no clusters (Figure 4.22b)

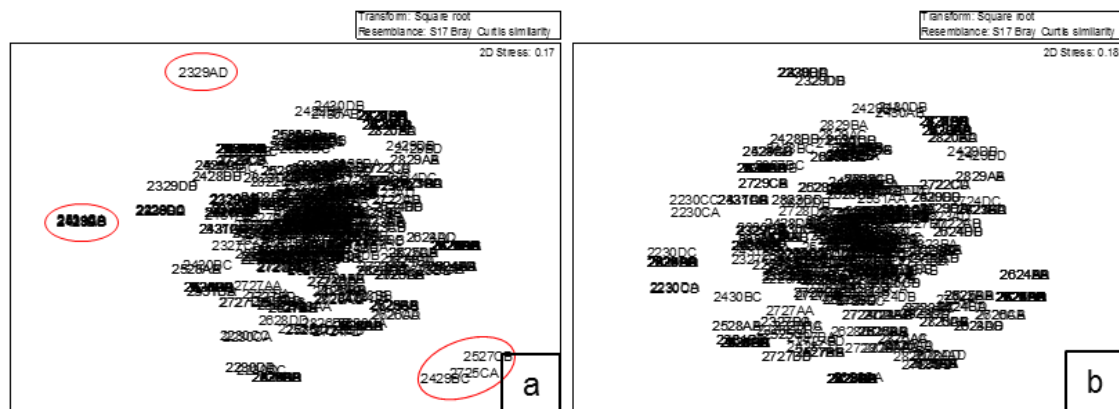


Figure 4.22: Initial standardized genera NMDS graph before outliers were removed (a) and after (b). Red circles (a) indicate the subjectively chosen outliers.

As a means to determine clusters, a Bray-Curtis similarity dendrogram was derived for standardized genus data to indicate clusters of QDGC's with similar species composition. The dendrogram analysis for Centroid Grid profile standardized genera had multiple divisions after a 40% resemblance, so that a 30% resemblance was chosen (Figure 4.24) – the same as with the non-standardized genera data. Similarity profile (SIMPROF) indicated definite objective clusters. The resemblance slice was applied to merge some SIMPROF clusters at the first definite junction (Figure 4.24). Clusters indicated by dendrogram and SIMPROF were inserted into the NMDS using *Factors* to create a final NMDS that contains nine clusters (Figure 4.23). The remaining QDGC clusters were inserted into PRIMER, and the resulting NMDS contains nine clusters (Figure 4.23).

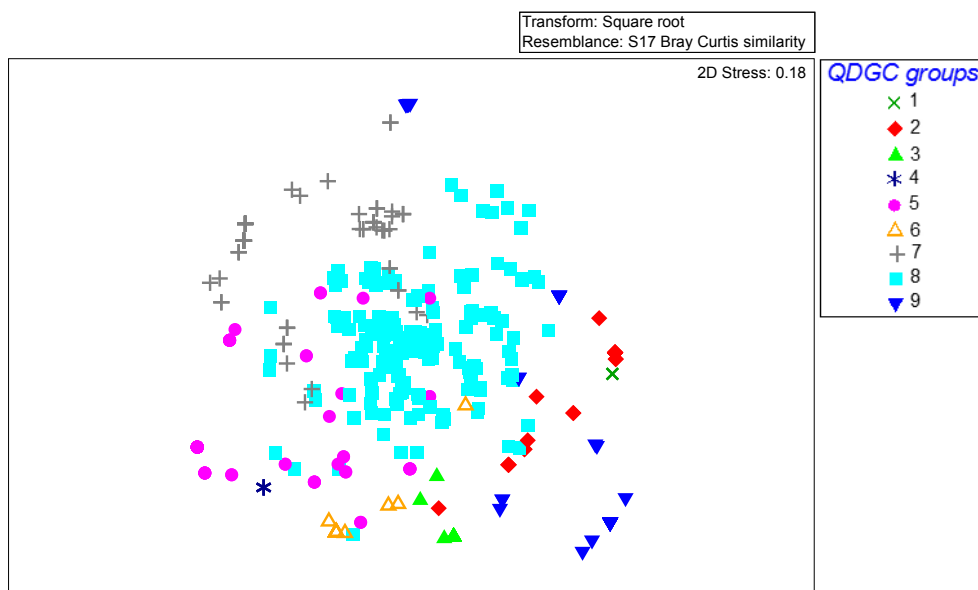


Figure 4.23: NMDS CG standardized genera, indicating clusters as determined by Bray-Curtis similarity and SIMPROF. Groups = clusters.

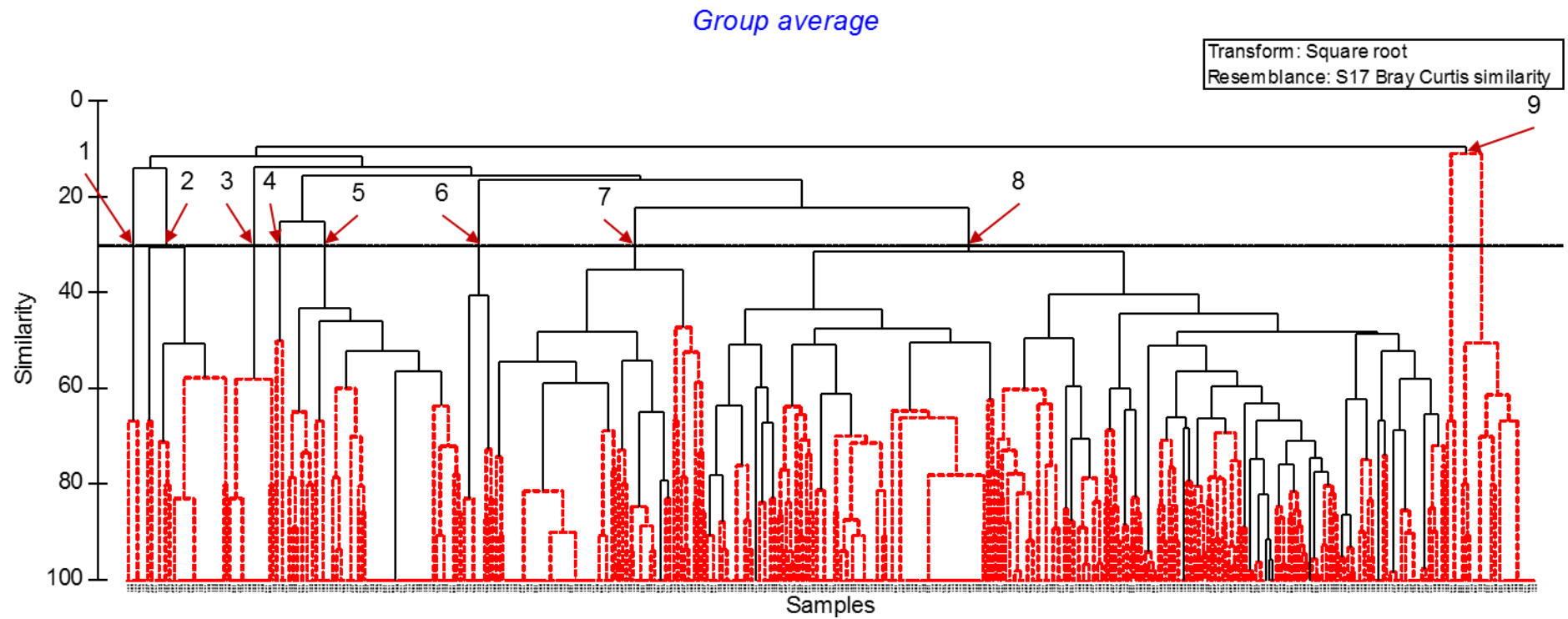


Figure 4.24: Dendrogram for standardized (CG) genera, indicating SIMPROF test (dotted red lines). Each sample is a QDGC. Resemblance slice (horizontal black line) was set at 30%. Clusters are indicated by numbers.

These CG standardized clusters for genera are indicated on a map of the study area by colouring each QDGC accordingly (Figure 4.25). Outliers were indicated, as it is possible for these QDGC's to form their own cluster.

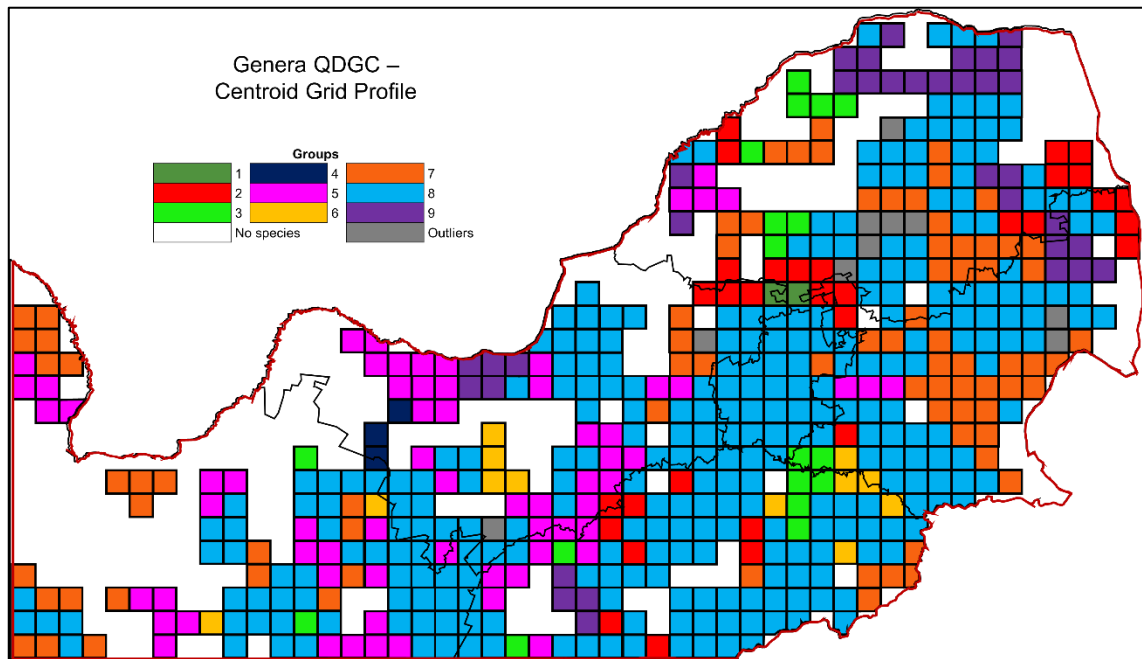


Figure 4.25: Distribution of CG standardized genera across the study area, indicating clusters with similar species composition as determined by Bray-Curtis similarity and SIMPROF. Groups = clusters.

The dominant species and genera for each of the non-standardized genera clusters was identified and is indicated in Table 4.7. Several clusters contained diagnostic species and genera that occurred regularly throughout the QDGC's of each cluster and was identified as dominant when occurring in QDGC's equal to or above the upper quartile value (Table 4.7).

Table 4.7: Genera clusters (standardized data) as determined by dendrogram, indicating number of QDGC's, specimens and the dominant species and genera present. Coloured squares coincide with clusters as indicated in figures 4.24, 4.25. Species and genera in bold occur throughout the most QDGC's within that cluster.

STANDARDIZED GENERA CLUSTERS						
Cluster number	Total number of grids	Mean	Standard deviation	Upper quartile (75%)	Number of individuals	Dominant species / genus
SPECIES						
1	2	2	0	2	4	<i>Sinapis alba</i> <i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>
2	32	4.31	4.55	5	56	<i>Erucastrum austroafricanum</i> <i>Rorippa fluviatilis</i> var. <i>caledonica</i> <i>Rorippa fluviatilis</i> var. <i>fluviatilis</i> <i>Rorippa madagascariensis</i>
3	18	3.43	3.6	4.5	24	<i>Erucastrum austroafricanum</i> <i>Erucastrum griquense</i>
4	3	3	0	3	9	<i>Coronopus integrifolius</i> <i>Raphanus raphanistrum</i> <i>Rapistrum rugosum</i>
5	68	5.62	10.83	5.75	146	<i>Capsella bursa-pastoris</i> <i>Coronopus didymus</i> <i>Coronopus integrifolius</i> <i>Erucastrum griquense</i> <i>Erucastrum strigosum</i> <i>Heliophila minima</i> <i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>
6	12	3.38	2.5	4.5	27	<i>Diplotaxis muralis</i> <i>Sisymbrium burchellii</i> var. <i>burchellii</i>
7	82	6.57	8.41	8.5	230	<i>Cardamine africana</i> <i>Erucastrum austroafricanum</i> <i>Heliophila carnos</i> <i>Heliophila deserticola</i> <i>Heliophila rigidiuscula</i> <i>Heliophila suavissima</i> <i>Heliophila trifurca</i> <i>Lepidium virginicum</i> <i>Nasturtium officinale</i>
8	290	26.54	3.12	43	2132	<i>Brassica rapa</i> <i>Capsella bursa-pastoris</i> <i>Coronopus integrifolius</i> <i>Erucastrum austroafricanum</i> <i>Erucastrum griquense</i> <i>Erucastrum strigosum</i> <i>Heliophila carnos</i> <i>Heliophila rigidiuscula</i> <i>Lepidium africanum</i> <i>Lepidium bonariense</i> <i>Lepidium schinzii</i> <i>Lepidium species</i> <i>Lepidium transvaalense</i> <i>Nasturtium officinale</i> <i>Raphanus raphanistrum</i> <i>Rorippa fluviatilis</i> var. <i>fluviatilis</i> <i>Rorippa nudiuscula</i> <i>Sisymbrium burchellii</i> var. <i>burchellii</i> <i>Sisymbrium capense</i>

							<i>Sisymbrium thellungi</i> <i>Sisymbrium turczaninowii</i>
	9	35	5.8	3.12	8	58	<i>Brassica elongata</i> <i>Brassica juncea</i> <i>Brassica rapa</i> <i>Lepidium africanum</i>
GENUS							
	1	2	2	0	2	4	<i>Sinapis</i> <i>Rorippa</i>
	2	32	6.88	10.71	4.75	56	<i>Erucastrum</i> <i>Rorippa</i>
	3	18	6	8.72	7	24	<i>Erucastrum</i>
	4	3	3	0	3	9	<i>Coronopus</i> <i>Raphanus</i> <i>Rapistrum</i>
	5	68	14.6	19.3	14	146	<i>Coronopus</i> <i>Erucastrum</i> <i>Heliophila</i> <i>Rorippa</i>
	6	12	5.4	5.03	8	27	<i>Diplotaxis</i> <i>Sisymbrium</i>
	7	82	19.17	34.88	19	230	<i>Cardamine</i> <i>Erucastrum</i> <i>Heliophila</i> <i>Lepidium</i>
	8	290	92.7	125.91	106.5	2132	<i>Coronopus</i> <i>Erucastrum</i> <i>Heliophila</i> <i>Lepidium</i> <i>Rorippa</i> <i>Sisymbrium</i>
	9	35	9.67	11.72	8.5	58	<i>Brassica</i> <i>Lepidium</i>

Standardized assemblages of genera were overlapped seasonal rainfall (SAWS 2015) (Figure 4.26a), mean rainfall over 50 years (Lynch 2004) (Figure 4.26b) and bioregion (Mucina & Rutherford 2006) (Figure 4.27) maps as a means of identifying possible factors influencing the distribution of the Brassicaceae.

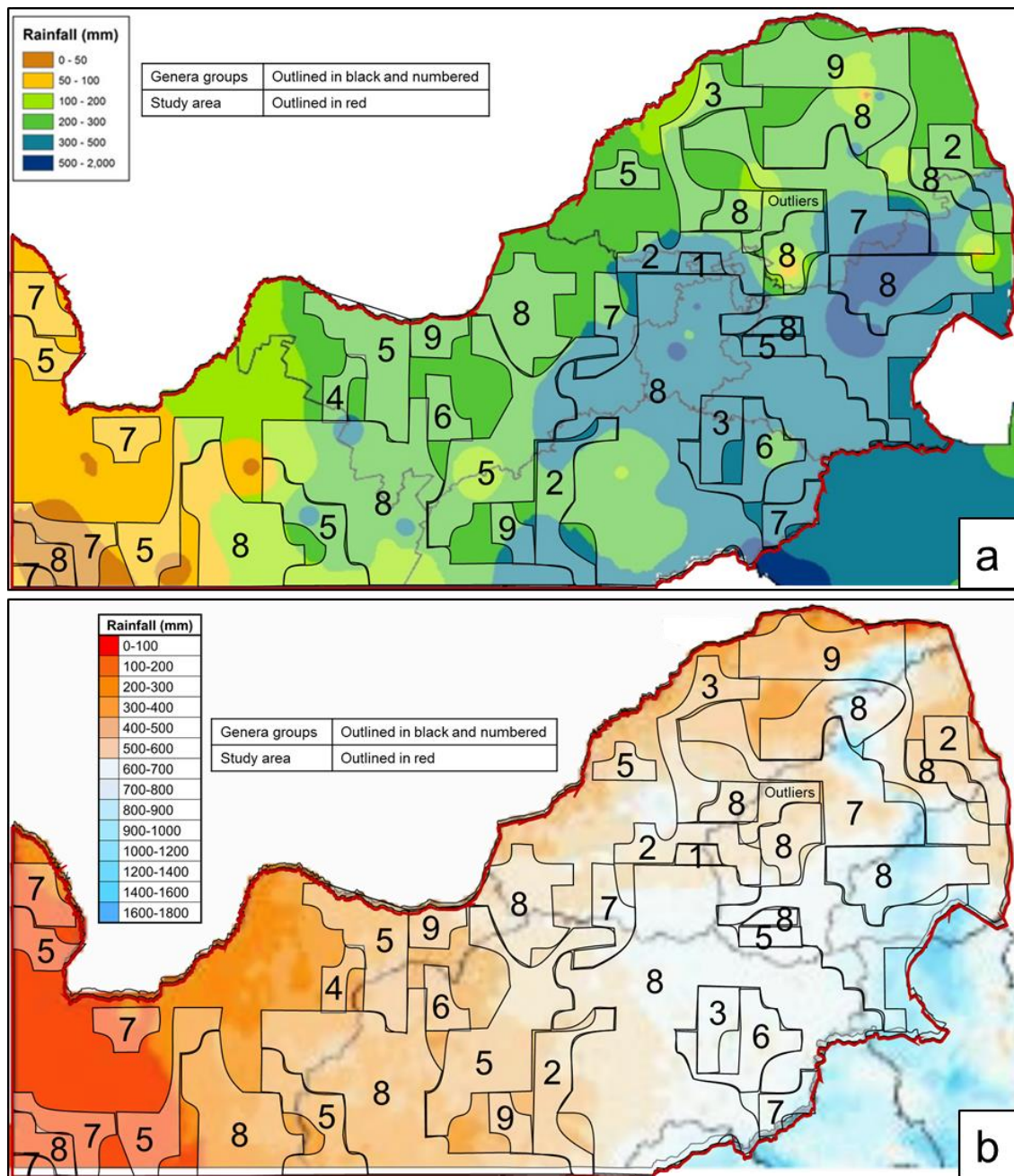


Figure 4.26: Seasonal rainfall (SAWS, 2015) (a) and mean rainfall between 1950 and 1999 (Lynch 2004) (b) overlapped with the distribution of standardized genera clusters (as determined by Bray-Curtis and SIMPROF). Clusters are outlined in black and numbered. Groups = Clusters.

None of the standardized genera clusters are present in any rainfall area (Figure 4.26) or single bioregion (Figure 4.27).

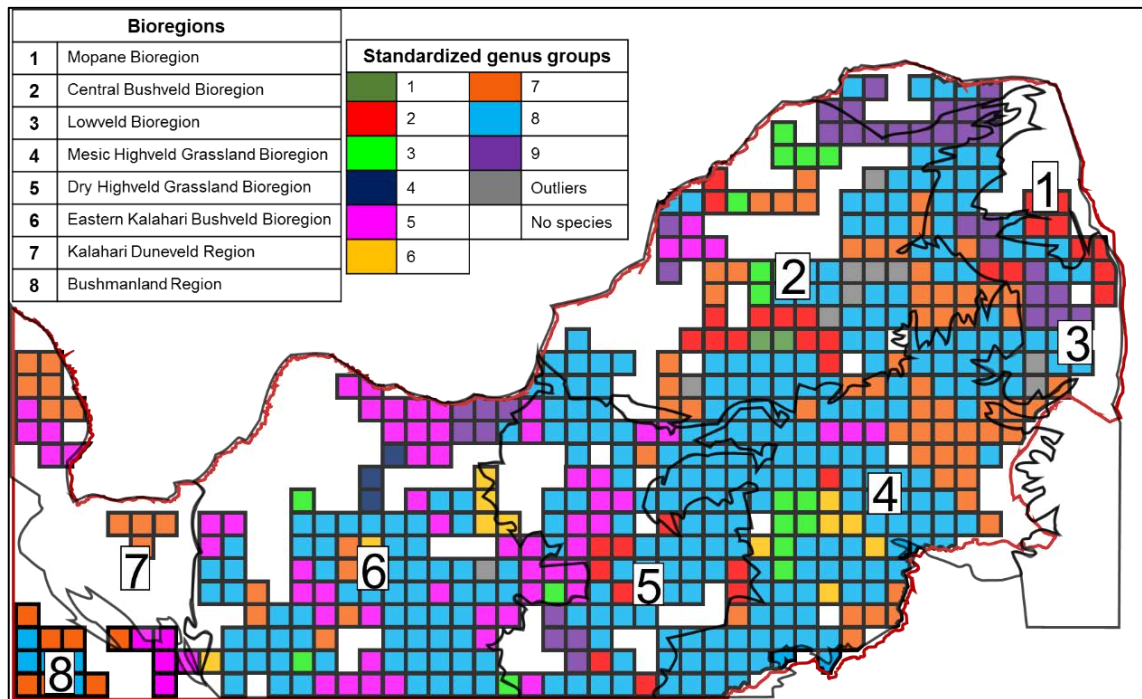


Figure 4.27: Distribution of standardized genera clusters, as determined by Bray-Curtis similarity and SIMPROF; indicated in coloured areas. It is overlapped with bioregions (Mucina & Rutherford 2006). Areas are outlined in black and numbered for identification. Groups = Clusters.

4.3.4 Tribe assemblages

4.3.4.1 Non-standardized

The non-standardized tribe QDGC's were subjected to NMDS in PRIMER. No outliers were present and the final NMDS graph indicated no clusters (Figure 4.28).

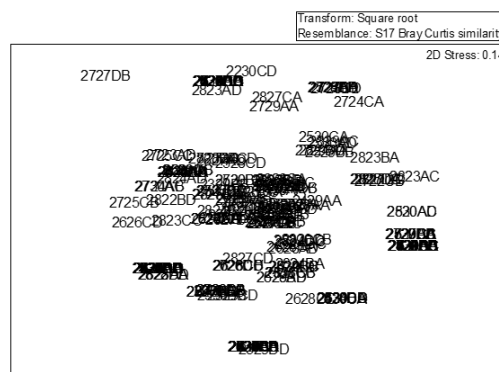


Figure 4.28: Initial non-standardized tribe NMDS graph, indicating no subjective outliers present and no clusters.

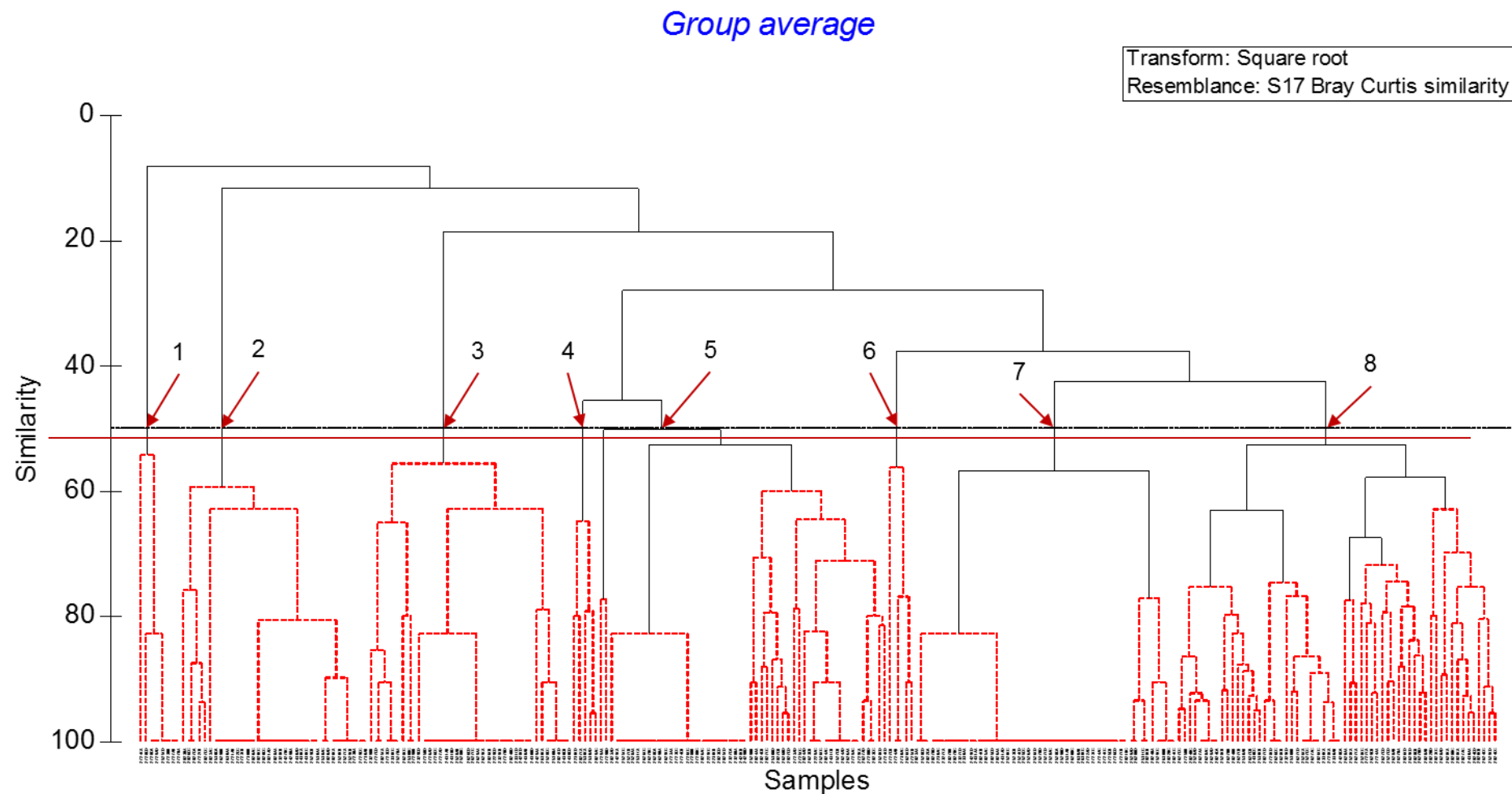


Figure 4.30: Dendrogram for non-standardized tribes, indicating SIMPROF test (dotted red lines). Each sample is a QDGC. Resemblance slice (horizontal black line) at 50%. Clusters are indicated by numbers.

These non-standardized tribes clusters are indicated on a map of the study area by colouring each QDGC accordingly (Figure 4.31).

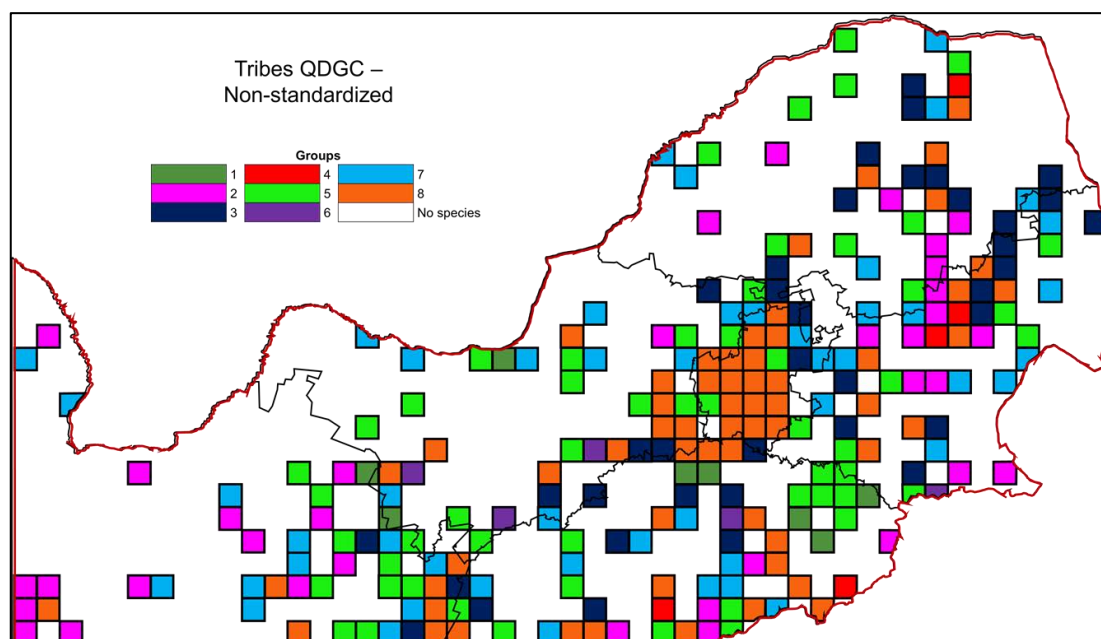


Figure 4.31: Distribution of non-standardized tribes across the study area, indicating clusters with similar species composition as determined by Bray-Curtis similarity and SIMPROF. Groups = clusters.

The dominant species and tribe was identified for each of the non-standardized tribe clusters and is indicated in Table 4.8. Several clusters contained diagnostic species and tribes that occurred regularly throughout the QDGC's of each cluster and was identified as dominant when occurring in QDGC's equal to or above the upper quartile value (Table 4.8).

Table 4.8: Tribe clusters (non-standardized data) as determined by dendrogram, indicating number of QDGC's, specimens and the dominant species and tribe present. Coloured squares coincide with clusters as indicated in figures 4.30, 4.31. Species and tribes present throughout the most QDGC's in a single cluster is indicated in bold.

NON-STANDARDIZED TRIBE CLUSTERS						
Cluster number	Total number of grids	Mean	Standard deviation	Upper quartile (75%)	Number of individuals	Dominant species / tribe
SPECIES						
1	8	1.71	0.76	2	12	<i>Sisymbrium burchellii</i> var. <i>burchellii</i> <i>Sisymbrium</i> species <i>Sisymbrium thellungii</i> <i>Sisymbrium turczaninowii</i>
2	35	4.39	6.14	4.75	79	<i>Heliophila carnosa</i> <i>Heliophila deserticola</i> <i>Heliophila rigidiuscula</i> <i>Heliophila suavissima</i>

							<i>Heliophila trifurca</i>
	3	38	4.39	6.17	7	102	Cardamine Africana <i>Heliophila rigidiuscula</i> <i>Nasturtium officinale</i> <i>Rorippa fluviatilis</i> var. <i>caledonica</i> <i>Rorippa fluviatilis</i> var. <i>fluviatilis</i> <i>Rorippa nudiuscula</i>
	4	5	3.58	5.62	3.25	43	<i>Capsella bursa-pastoris</i> Heliophila rigidiuscula <i>Raphanus raphanistrum</i>
	5	54	4.17	4.32	5	175	<i>Coronopus integrifolius</i> <i>Diplotaxis muralis</i> Erucastrum austroafricanum <i>Erucastrum griquense</i> <i>Erucastrum strigosum</i> <i>Heliophila suavissima</i> <i>Lepidium africanum</i> <i>Lepidium bonariense</i> <i>Raphanus raphanistrum</i> <i>Rapistrum rugosum</i> <i>Sisymbrium burchellii</i> var. <i>burchellii</i> <i>Sisymbrium turczaninowii</i>
	6	5	2.14	1.46	2.5	15	Capsella bursa-pastoris <i>Coronopus integrifolius</i>
	7	49	4.1	5.26	4	86	Coronopus integrifolius <i>Heliophila carnosa</i> <i>Heliophila rigidiuscula</i> <i>Lepidium africanum</i> <i>Lepidium bonariense</i> <i>Lepidium virginicum</i>
	8	60	12.31	16.75	17	788	<i>Capsella bursa-pastoris</i> <i>Coronopus integrifolius</i> <i>Diplotaxis muralis</i> <i>Erucastrum austroafricanum</i> <i>Heliophila carnosa</i> <i>Heliophila rigidiuscula</i> Lepidium africanum <i>Lepidium bonariense</i> <i>Lepidium transvaalense</i> <i>Lepidium virginicum</i> <i>Nasturtium officinale</i> <i>Raphanus raphanistrum</i> <i>Rorippa fluviatilis</i> var. <i>fluviatilis</i> <i>Rorippa nudiuscula</i> <i>Sisymbrium orientale</i> <i>Sisymbrium thellungi</i> <i>Sisymbrium turczaninowii</i>
TRIBE							
	1	8	6	5.66	8	12	Sisymbrieae
	2	35	26.33	36.25	38.5	79	Heliophileae
	3	38	23	22.72	26	102	Cardamineae
	4	5	10.75	11.18	15.5	43	Heliophileae
	5	54	25	28.08	35	175	Brassicaceae Lepidieae
	6	5	5	3	6.5	15	Lepidieae
	7	49	28.67	31.18	40.5	86	Heliophileae
	8	60	77.44	90.18	148	788	Brassicaceae Cardamineae Lepideae

4.3.4.2 Standardized

The standardized tribe QDGC's were subjected to NMDS in PRIMER. No outliers were present and the final NMDS graph indicated no clusters (Figure 4.32)

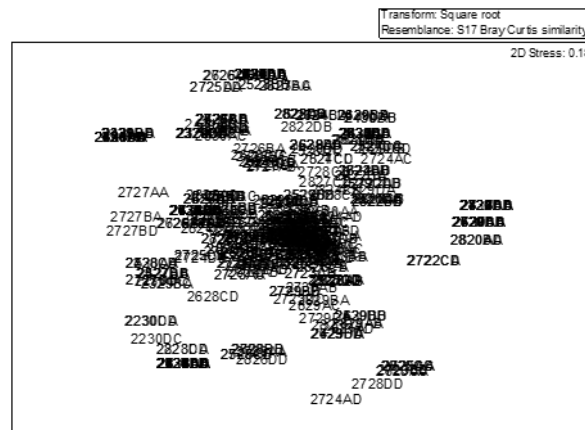


Figure 4.32: Initial standardized tribe NMDS graph, indicating no subjective outliers present and no clusters.

As a means to determine clusters, a Bray-Curtis similarity dendrogram was derived for standardized tribe data to indicate clusters of QDGC's with similar species composition. A 50% resemblance was chosen for non-standardized tribe dendrogram analysis (Figure 4.33). The QDGC clusters, indicating similar species composition, were inserted into PRIMER and the resulting NMDS contains nine clusters (Figure 4.33). Similarity profile (SIMPROF) indicated definite objective clusters. The resemblance slice was applied to merge some SIMPROF clusters at the first definite junction (Figure 4.33). Clusters indicated by dendrogram and SIMPROF were inserted into the NMDS using Factors to create a final NMDS that contains nine clusters (Figure 4.34). The remaining QDGC clusters were inserted into PRIMER, and the resulting NMDS contains nine clusters (Figure 4.34).

Group average

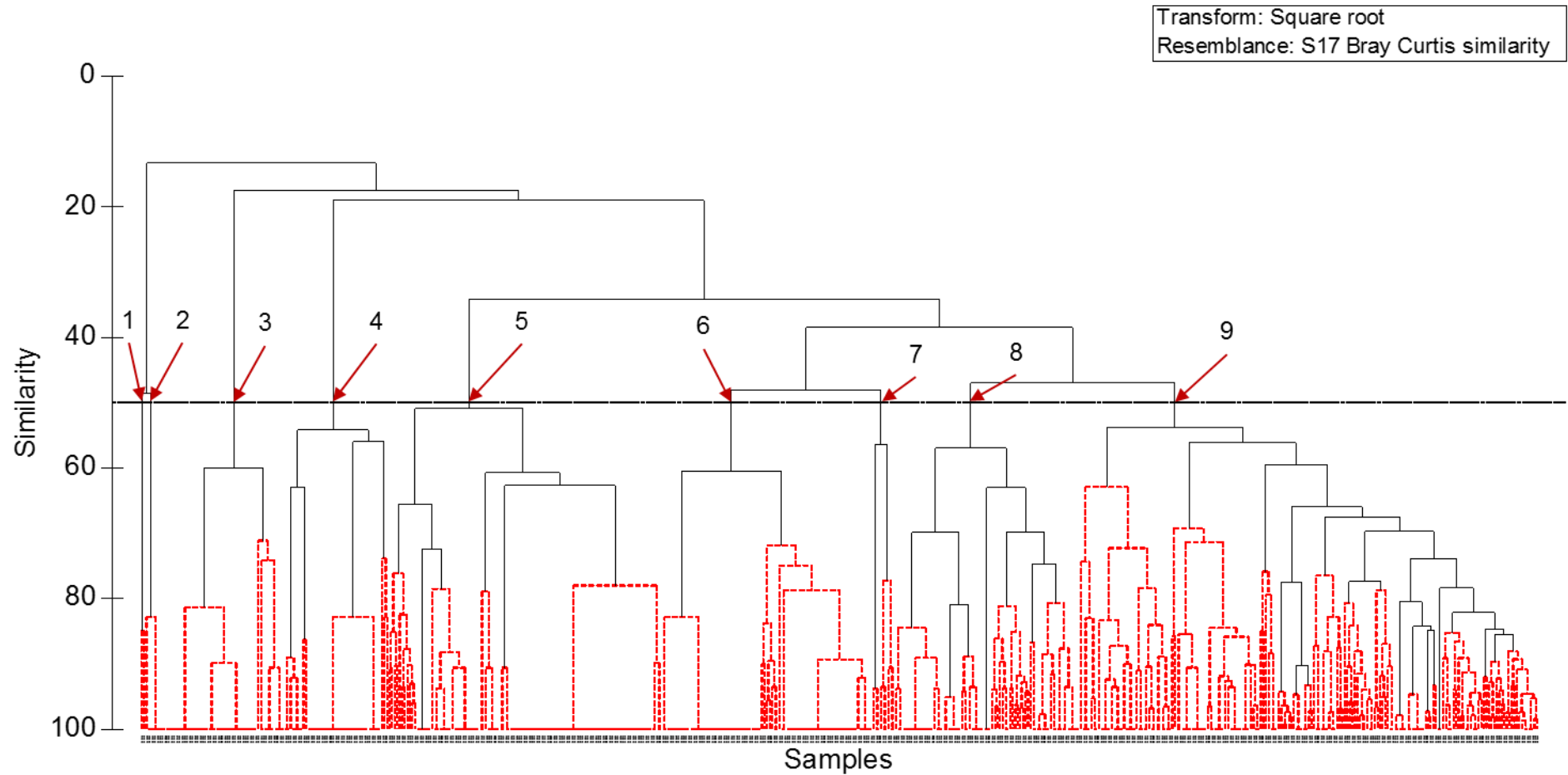


Figure 4.33: Dendrogram for standardized (CG) tribes, indicating SIMPROF test (dotted red lines). Each sample is a QDGC. Resemblance slice (horizontal solid red line) at 50%. Clusters are indicated by numbers.

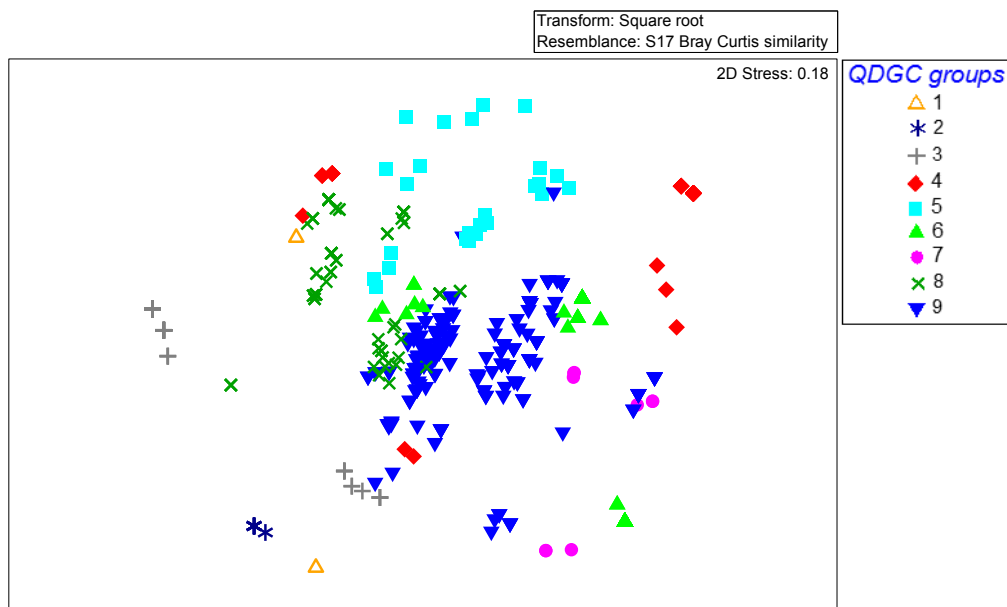


Figure 4.34: NMDS CG standardized tribes, indicating clusters as determined by Bray-Curtis similarity and SIMPROF. Groups = clusters.

These CG standardized tribe clusters are indicated on a map of the study area by colouring each QDGC accordingly (Figure 4.35).

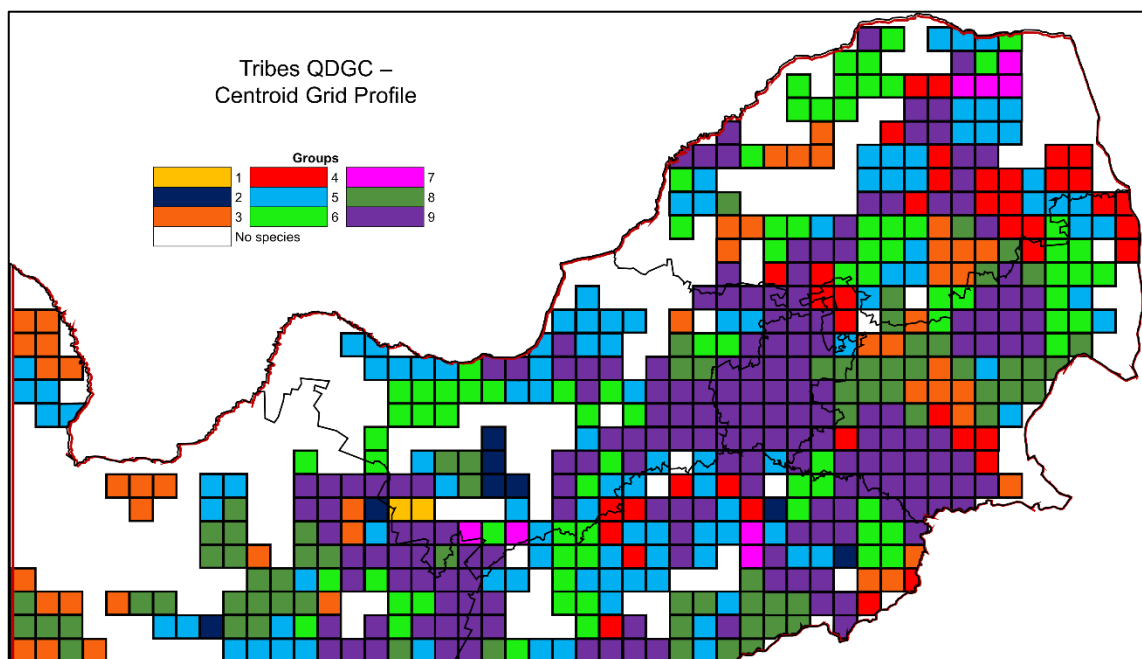


Figure 4.35: Distribution of CG standardized tribes across the study area, indicating clusters with similar species composition as determined by Bray-Curtis similarity and SIMPROF. Groups = clusters.

The dominant species and tribe for each of the standardized tribe clusters was identified and is indicated in Table 4.9. Several clusters contained diagnostic species and tribes that occurred regularly throughout the QDGC's of each cluster and was identified as dominant when occurring in QDGC's equal to or above the upper quartile value (Table 4.9).

Table 4.9: Tribe clusters (standardized data) as determined by dendrogram, indicating number of QDGC's, specimens and the dominant species and tribe present. Coloured squares coincide with clusters as indicated in figure 4.34, 4.35.

STANDARDIZED TRIBE CLUSTERS						
Cluster number	Total number of grids	Mean	Standard deviation	Upper quartile (75%)	Number of individuals	Dominant species / tribe
SPECIES						
1	2	1.8	0.45	2	9	<i>Matthiola</i> species <i>Matthiola torulosa</i> <i>Sisymbrium burchellii</i> var. <i>burchellii</i> <i>Sisymbrium</i> species
2	8	2.67	2.89	3.5	8	<i>Sisymbrium burchellii</i> var. <i>burchellii</i>
3	47	4.84	5.17	5.5	92	<i>Heliophila carnos</i> <i>Heliophila deserticola</i> <i>Heliophila rigidiuscula</i> <i>Heliophila suavissima</i> <i>Heliophila trifurca</i>
4	41	3.68	3.62	5	81	<i>Cardamine africana</i> <i>Heliophila rigidiuscula</i> <i>Nasturtium officinale</i> <i>Rorippa fluviatilis</i> var. <i>caledonica</i> <i>Rorippa fluviatilis</i> var. <i>fluviatilis</i> <i>Rorippa madagascariensis</i> <i>Rorippa nudiuscula</i>
5	108	7.9	9.29	7	238	<i>Cardamine africana</i> <i>Coronopus integrifolius</i> <i>Lepidium africanum</i> <i>Lepidium bonariense</i> <i>Lepidium</i> species <i>Lepidium virginicum</i> <i>Nasturtium officinale</i> <i>Rorippa fluviatilis</i> var. <i>caledonica</i> <i>Rorippa fluviatilis</i> var. <i>fluviatilis</i> <i>Rorippa nudiuscula</i> <i>Sisymbrium burchellii</i> var. <i>burchellii</i> <i>Sisymbrium turczaninowii</i>
6	83	5.48	4.25	8	159	<i>Coronopus integrifolius</i> <i>Erucastum austroafricanum</i> <i>Erucastum griquense</i> <i>Erucastum strigosum</i> <i>Lepidium africanum</i> <i>Lepidium bonariense</i> <i>Raphanus raphanistrum</i> <i>Sinapis arvensis</i>
7	9	2.79	1.12	3	39	<i>Brassica juncea</i> <i>Brassica rapa</i> <i>Capsella bursa-pastoris</i> <i>Coronopus integrifolius</i> <i>Erucastum austroafricanum</i>

							<i>Erucastum griquense</i> <i>Lepidium africanum</i> <i>Matthiola species</i> <i>Turritis glabra</i>
	8	73	8.45	7.42	11	372	<i>Coronopus didymus</i> <i>Coronopus integrifolius</i> <i>Erucastum austroafricanum</i> <i>Heliophila carnososa</i> <i>Heliophila minima</i> <i>Heliophila rigidiuscula</i> <i>Heliophila species</i> <i>Heliophila suavissima</i> <i>Heliophila trifurca</i> <i>Lepidium africanum</i> <i>Lepidium bonariense</i> <i>Sisymbrium burchellii</i> var. <i>burchellii</i> <i>Sisymbrium capense</i>
	9	181	22.29	22.78	35.75	1695	<i>Brassica rapa</i> <i>Capsella bursa-pastoris</i> <i>Coronopus integrifolius</i> <i>Diplotaxis muralis</i> <i>Erucastum austroafricanum</i> <i>Erucastum griquense</i> <i>Erucastum strigosum</i> <i>Heliophila rigidiuscula</i> <i>Lepidium africanum</i> <i>Lepidium bonariense</i> <i>Lepidium transvaalense</i> <i>Nasturtium officinale</i> <i>Raphanus raphanistrum</i> <i>Rorippa fluviatilis</i> var. <i>fluviatilis</i> <i>Rorippa nudiuscula</i> <i>Sisymbrium burchellii</i> var. <i>burchellii</i> <i>Sisymbrium capense</i> <i>Sisymbrium thellungi</i> <i>Sisymbrium turczaninowii</i>
TRIBE							
	1	2	3	1.73	4	9	Anchonieae
	2	8	8	–	8	8	Sisymbrieae
	3	47	23	36.94	27.75	92	Heliophieae
	4	41	15.4	21.54	14	81	Cardamineae Heliophileae
	5	108	34	51.55	32.5	238	Cardamineae Lepidieae
	6	83	39.75	43.53	63	159	Brassiceae
	7	9	7.8	5.36	9	39	Camelineae Brassiceae
	8	73	53.14	55.46	84	372	Heliophieae Lepidieae
	9	181	169.5	178.29	267	1695	Brassiceae Cardamineae Lepidieae

Standardized assemblages of tribes were overlapped with bioregion (Mucina & Rutherford 2006) (Figure 4.36), seasonal rainfall (SAWS 2015) (Figure 4.37a) and mean rainfall over 50 years (Lynch 2004) (Figure 4.37b) maps as a means of identifying possible factors influencing the distribution of the Brassicaceae.

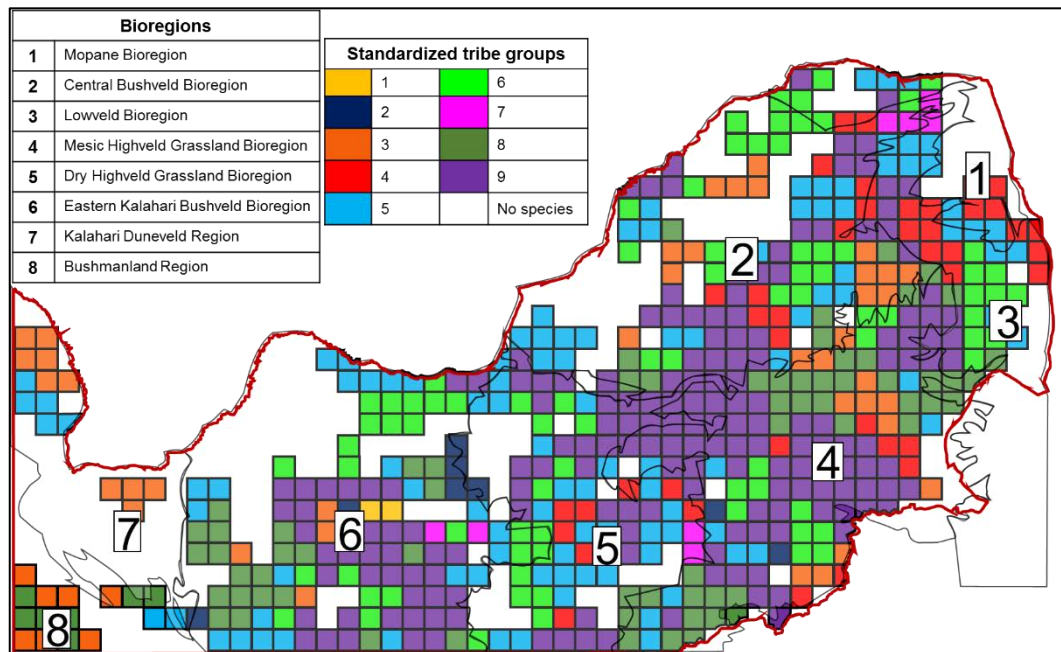


Figure 4.36: Distribution of standardized tribe clusters, as determined by Bray-Curtis similarity and SIMPROF; indicated in coloured areas. It is overlapped with bioregions (Mucina and Rutherford, 2006); areas outlined in black and numbered for identification. Groups = clusters.

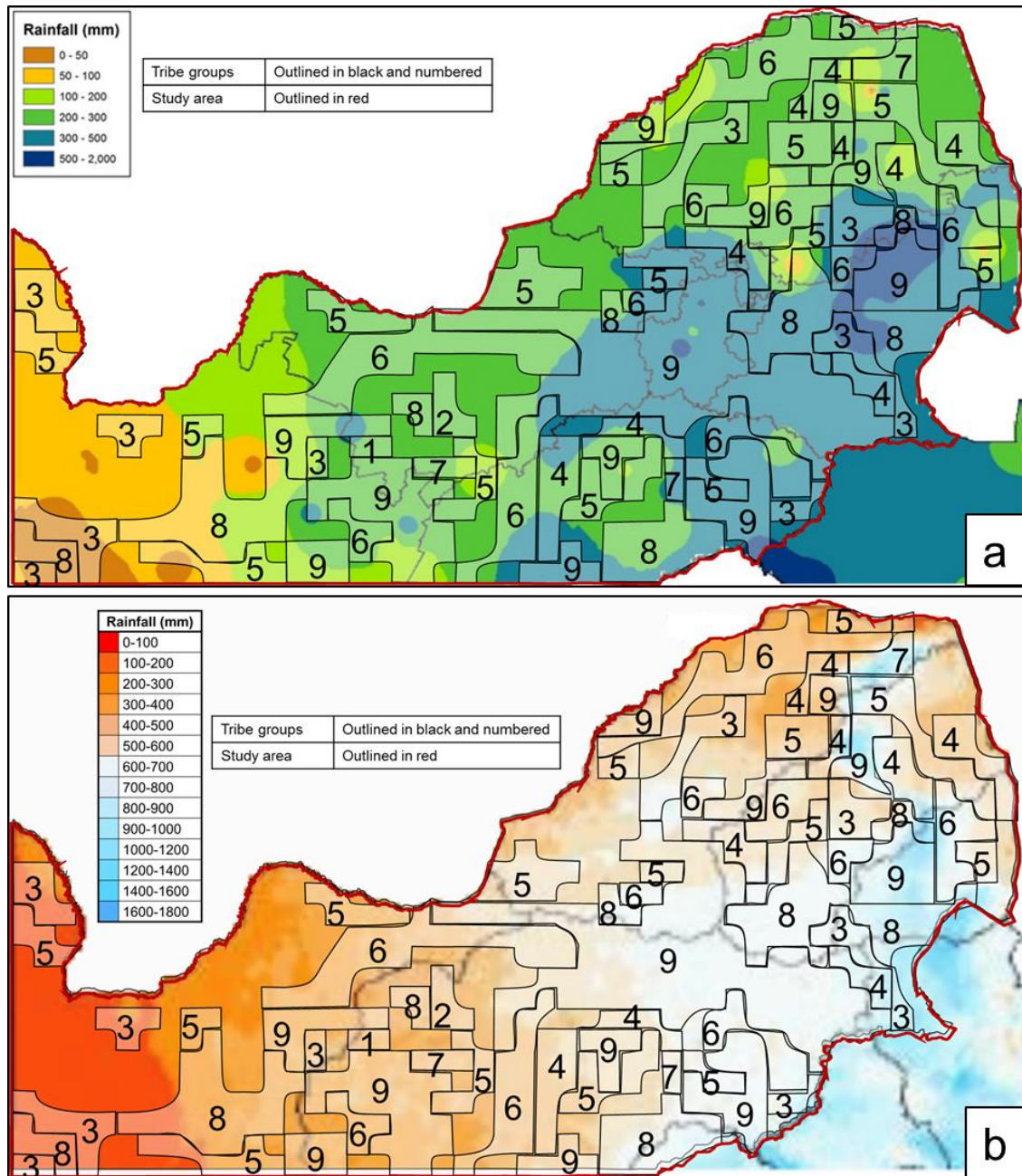


Figure 4.37: Seasonal rainfall (SAWS 2015) (a) and mean rainfall between 1950 and 1999 (Lynch 2004) (b) overlapped with the distribution of standardized tribe clusters (as determined by Bray-Curtis and SIMPROF); clusters outlined in black and numbered. Groups = clusters.

4.3.5 Similarity between clusters

The non-standardized species data set had the lowest R value of the six data sets (0.5016) and the standardized tribe distribution presented the highest value (0.8245) (Table 4.10). Lower R-values indicate less distinctiveness between the QDGC clusters, while increasing R-values is indicative of increasing distinctiveness. At the species level clusterings were thus much less

pronounced than at higher hierarchical levels, and non-standardized sets have much weaker distinctiveness between clusters than the Centroid Grid profile data.

Table 4.10: ANOSIM R values for each of the six data sets.

	Non-standardized	Centroid Grid profile
Species	0.5016	0.626
Genera	0.7027	0.768
Tribes	0.6503	0.8245

SIMPER analyses indicated that the average dissimilarity between clusters in each of the six different data sets were relatively high, with the non-standardized species data set presenting the highest overall dissimilarity with 92.24% (Table 4.11) and the standardized tribe data set having the lowest dissimilarity (68.14%, Table 4.11).

Table 4.11: SIMPER average dissimilarity percentages across each of the six data sets.

	Non-standardized	Centroid Grid profile
Species	92.24	89.70
Genera	81.17	77.51
Tribes	72.87	68.14

Table 4.12: Average dissimilarity (AD) and % contribution to average dissimilarity (Table 4.11) (C) of the most significant species, genera and tribes within the six data sets.

	Non-standardized		Standardized	
	AD	C	AD	C
Species				
<i>Heliophila rigidiuscula</i>	9.288	9.891	4.346	4.845
<i>Lepidium africanum</i>	7.339	7.815	5.594	6.237
<i>Coronopus integrifolius</i>	6.578	7.005	5.809	6.476
<i>Erucastrum austroafricanum</i>	4.716	5.023	4.149	4.626
Genera				
<i>Lepidium</i>	15.14	18.65	13.79	17.79
<i>Heliophila</i>	14.62	18.01	12.63	16.3
<i>Sisymbrium</i>	8.77	10.81	8.384	10.82
<i>Erucastrum</i>	9.058	11.16	8.084	10.43

Tribes				
Lepidieae	18.46	25.34	16.16	23.72
Brassicaceae	16.08	22.06	15.91	23.34
Heliophilleae	14.72	20.2	12.6	18.49
Cardamineae	12.27	16.84	10.96	16.09

Heliohila rigidiuscula had the highest average dissimilarity (9.288%), contributing 9.891% to the non-standardized species distribution of the Brassicaceae across the study area (Table 4.12). It is followed by *Lepidium africanum*, *Coronopus integrifolius*, and *Erucastrum austroafricanum* (Table 4.12). These percentages of species that contributed the most to the overall dissimilarity of the non-standardized Brassicaceae distribution are very low (Table 4.12), and coincide with the overall dissimilarity percentage (Table 4.11) and overall R value obtained (Table 4.10) for the non-standardized species data set.

4.3.6 Reproductively compatible species

Brassica napus has been cultivated in 2726BC, 2728CD, 2828AA and 2828CB during previous years (W. Jonker, J. Vermeulen and B. van Rensburg pers com). Eight specimens of other Brassicaceae species were collected in the same QDGC's and 69 in the surrounding QDGC's. Among these were four specimens of three species (*Diplotaxis muralis*, *Raphanus raphanistrum* and *Rapistrum rugosum*) that are confirmed to be reproductively compatible with *B. napus* as confirmed by previous studies (Table 4.13).

Table 4.13: Species that are present in the study area and reproductively compatible with *Brassica napus* as indicated by global research. *Species growing in QDGC's surrounding *B. napus* cultivation areas of the northern provinces of South Africa.

nr	Species	Taxa Status	Reference/s
Species that are compatible with <i>B. napus</i>			
1	<i>Brassica juncea</i>	Naturalised	Aono <i>et al.</i> 2006; Scheffler & Dale 1994
2	<i>Brassica nigra</i>	Naturalised	FitzJohn <i>et al.</i> 2007
3	<i>Brassica rapa</i>	Naturalised	Allainguillaume <i>et al.</i> 2006; Allender & King 2010; Anjum <i>et al.</i> 2012; Aono <i>et al.</i> 2006; FitzJohn <i>et al.</i> 2007; Scheffler & Dale 1994; Warwick <i>et al.</i> 2003
4	* <i>Diplotaxis muralis</i>	Naturalised	FitzJohn <i>et al.</i> 2007
5	<i>Eruca sativa</i>	Naturalised	Fahleson <i>et al.</i> 1997
6	* <i>Raphanus raphanistrum</i>	Naturalised	FitzJohn <i>et al.</i> 2007; Lefol <i>et al.</i> 1997; Scheffler & Dale 1994; Warwick <i>et al.</i> 2003
7	* <i>Rapistrum rugosum</i>	Naturalised	FitzJohn <i>et al.</i> 2007

8	<i>Sinapis alba</i>	Naturalised	FitzJohn <i>et al.</i> 2007
9	<i>Sinapis arvensis</i>	Naturalised	FitzJohn <i>et al.</i> 2007; Moyes and Barriers 2002; Warwick <i>et al.</i> 2003
<i>B. rapa</i> as genetic bridge species to <i>B. napus</i>			
1	<i>Brassica juncea</i>	Naturalised	Aono <i>et al.</i> 2006, FitzJohn <i>et al.</i> 2007; Scheffler & Dale 1994
2	<i>Brassica nigra</i>	Naturalised	FitzJohn <i>et al.</i> 2007
3	<i>Raphanus sativus</i>	Naturalised	FitzJohn <i>et al.</i> 2007
<i>B. juncea</i> as genetic bridge species to <i>B. napus</i>			
1	<i>Brassica nigra</i>	Naturalised	FitzJohn <i>et al.</i> 2007
2	<i>Brassica rapa</i>	Naturalised	Allainguillaume <i>et al.</i> 2006; Allender & King 2010; Anjum <i>et al.</i> 2012; Aono <i>et al.</i> 2006; FitzJohn <i>et al.</i> 2007; Scheffler & Dale 1994; Warwick <i>et al.</i> 2003

4.3.6.1 Dominant reproductively compatible species distribution

Ten Brassicaceae species was regarded as reproductively compatible with *B. napus* (Table 4.13). *Brassica rapa* is present in cluster two, *Diplotaxis muralis* and *Rapistrum rugosum* in cluster five, and *Raphanus raphanistrum* in cluster six of the non-standardized Brassicaceae species distribution (Table 4.4). Cluster two is distributed across twelve QDGC's and three of its five species is considered dominant (Appendix D; Figure 4.11; Table 4.4). Its few dominant species (7) is low when compared to the total species (27) and is supported by the high standard deviation (8.61) which states that the average dissimilarity between any given species and the total average per species in the cluster (5.96) is 8.61. Clusters two and five are thus considered adequate to represent the distribution of the reproductively compatible relatives that are present therein. Cluster six contains 973 specimens and 71 species that are distributed across 127 QDGC's (Appendix D), its standard deviation is very high (22.57) compared to the mean (13.73), and it only contains a single dominant reproductively compatible relative (Table 4.4) so that this cluster was deemed to not be diagnostically satisfactory of the distribution of non-standardized reproductively compatible relatives within the Brassicaceae.

Brassica rapa, *Diplotaxis muralis* and *Raphanus raphanistrum* are dominant in cluster 12 of the standardized species distribution of the Brassicaceae. Cluster 12, much like cluster six of the non-standardized species distribution, contains many individuals (1802) of many species (69) distributed across many QDGC's (277) (Appendix D; Table 4.5). It further has a very high standard deviation (29.78) when compared to the low average of individuals per species (26.13), presenting a low upper quartile (39) (Table 4.5) and was consequently not deemed to be adequate to represent the distribution of the reproductively compatible relatives. *Brassica rapa* is further present in cluster one, *Raphanus raphanistrum* in cluster six. *Rapistrum rugosum*

in cluster 10, and *Sinapis arvensis* in cluster five. Cluster 10 is not a satisfactory diagnostic group (total individuals = 627; QDGC's = 138) (Table 4.5). Clusters one, five and six contain few species (4, 9 and 2 respectively) across few QDGC's (9, 9 and 4 respectively) (Figure 4.15; Table 4.5). The upper quartile of each of these clusters are high (5.75, 7.5 and 4 respectively) when compared to the mean (4, 6 and 4 respectively) so that these clusters were considered diagnostically satisfactory for indication of the standardized distribution of reproductively compatible species. The standardized distribution of species indicate overall better clusters than the non-standardized species distribution.

4.3.6.2 Dominant reproductively compatible genera distribution

When comparing the non-standardized distribution (obtained from herbaria records) of known reproductively compatible genera (Table 4.13) to where *B. napus* has previously been cultivated, *Eruca* (Figure 4.5f) and *Sinapis* (Figure 4.5o) are the least likely to hybridize since these species only occur in the Gauteng province. *Brassica* (Figure 4.5a), *Diplotaxis* (Figure 4.5e), *Erucastrum* (Figure 4.5g), and *Rapistrum* (Figure 4.5m) occurs in the same area albeit to a lesser extent, with *Raphanus* (Figure 4.5l) being the most frequent reproductively compatible genus.

With the exception of *Erucastrum*, these genera make up the species that are reproductively compatible with *B. napus* (Table 4.13). *Brassica* is the only reproductively compatible genus that is dominant in a cluster (cluster four) of the non-standardized genus distribution (Table 4.6). The standardized distribution increased the dominance of the reproductively compatible relatives so that *Brassica* is present in cluster nine that presents an upper quartile (8.5) that is lower than the mean (9.67), *Diplotaxis* in cluster six that contains only 27 individuals across 12 QDGC's, *Raphanus* and *Rapistrum* in cluster four, and *Sinapis* of cluster one, that both have a standard deviation of 0 (Table 4.7). Clusters one and four consist of two and three QDGC's respectively (Table 4.7). Clusters six and nine, consisting of 12 and 35 QDGC's respectively, is distributed along the north-east, east and south-east of the study area (Figure 4.25; Table 4.7). The standardized distribution of genera indicate overall better clusters than the non-standardized genera distribution.

4.3.6.3 Dominant reproductively compatible tribe distribution

All the reproductively compatible relatives are of tribe Brassiceae. The Brassiceae are dominant in clusters five and eight of the non-standardized distribution (Table 4.8). The upper quartile of cluster five is very high (35) when compared to the mean (25) of the total individuals within the cluster (175) so that the dominance of two of the three tribes within the cluster is expected. The

Brassicaceae is distributed throughout the most QDGC's in cluster five (Appendix D; Table 4.8). Cluster eight has a high standard deviation (90.18) when compared to the average individuals per species (77.44) and is thus not as diagnostic to the distribution of the Brassicaceae as cluster five. Both these clusters are dispersed along central and south-east of the study area (Figure 4.31).

The Brassicaceae is dominant in clusters six, seven and nine of the standardized distribution of the Brassicaceae tribe clusters (Table 4.9). Cluster nine, consisting of 1695 individuals, occurs throughout a large area of 181 QDGC's and was consequently deemed less diagnostic than clusters six and seven (Figure 4.35; Table 4.9). The single dominant tribe of the four tribes present within the cluster (Appendix D), the Brassicaceae, is included in an upper quartile of 63 (Table 4.9). When compared to the number of individuals present (159) and the mean (39.75) this cluster is deemed as adequate representation of the Brassicaceae. Cluster seven consists of only two tribes that are both dominant, and nine QDGC's (Table 4.9) so that it may be considered to further contribute to the distribution of the reproductively compatible relatives. The standardized distribution of tribes indicate overall better clusters than the non-standardized tribe distribution.

4.3.6.4 Environmental factors

Each of the Brassicaceae distributions indicating presence of reproductively compatible species (non-standardized, Figure 4.38a, and standardized, Figure 4.38b) were overlaid onto bioregions (Figure 4.39) and rainfall (Figure 4.40). Transparent QDGC's contain no reproductively compatible species.

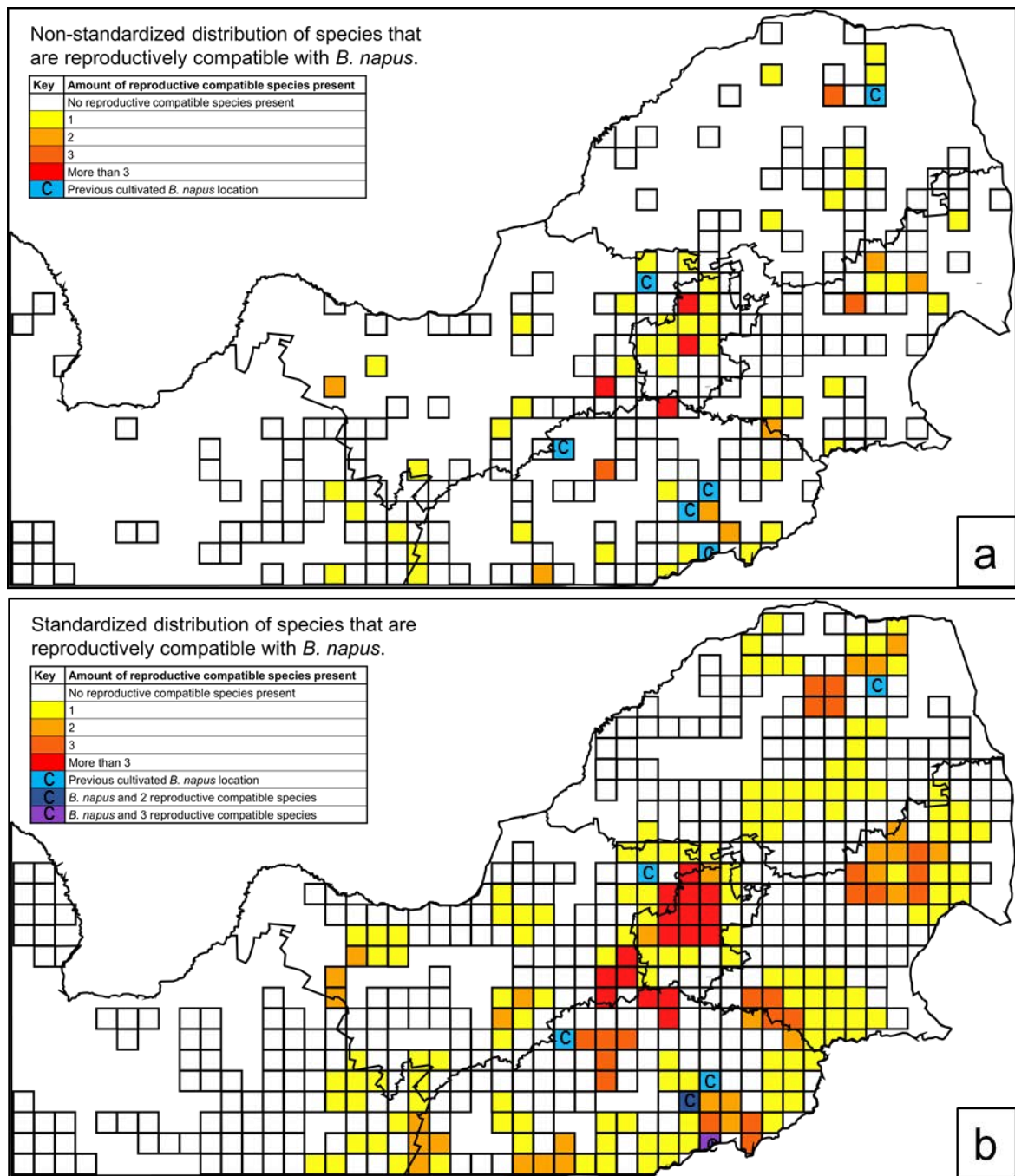


Figure 4.38: Non-standardized (a) and standardized (b) distribution of all species that are reproductively compatible with *B. napus* (Table 4.13).

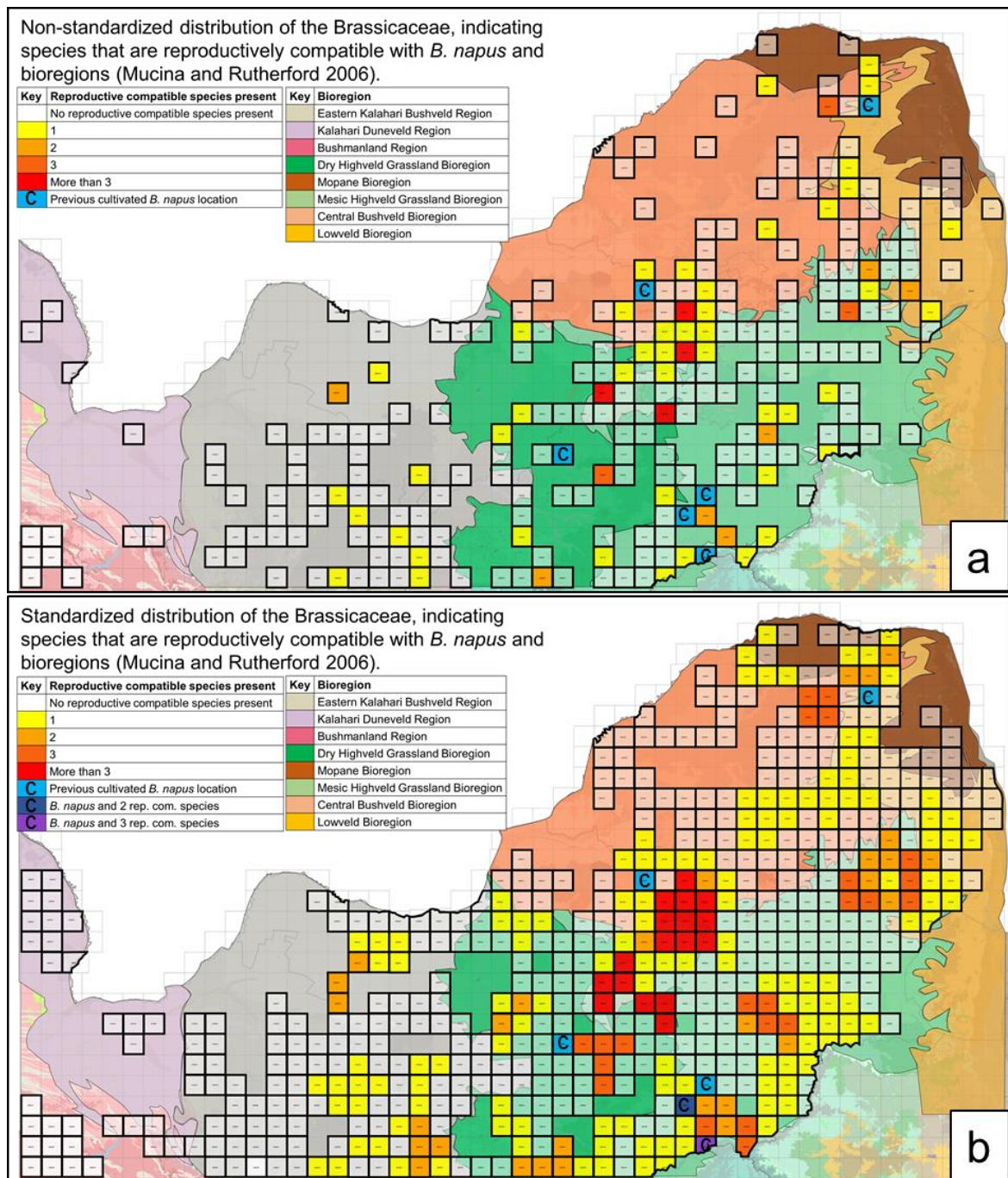


Figure 4.39: Non-standardized (a) and standardized (b) distribution of the Brassicaceae, indicating all species that are reproductively compatible with *B. napus* (Table 4.13) compared to bioregions (Mucina & Rutherford 2006).

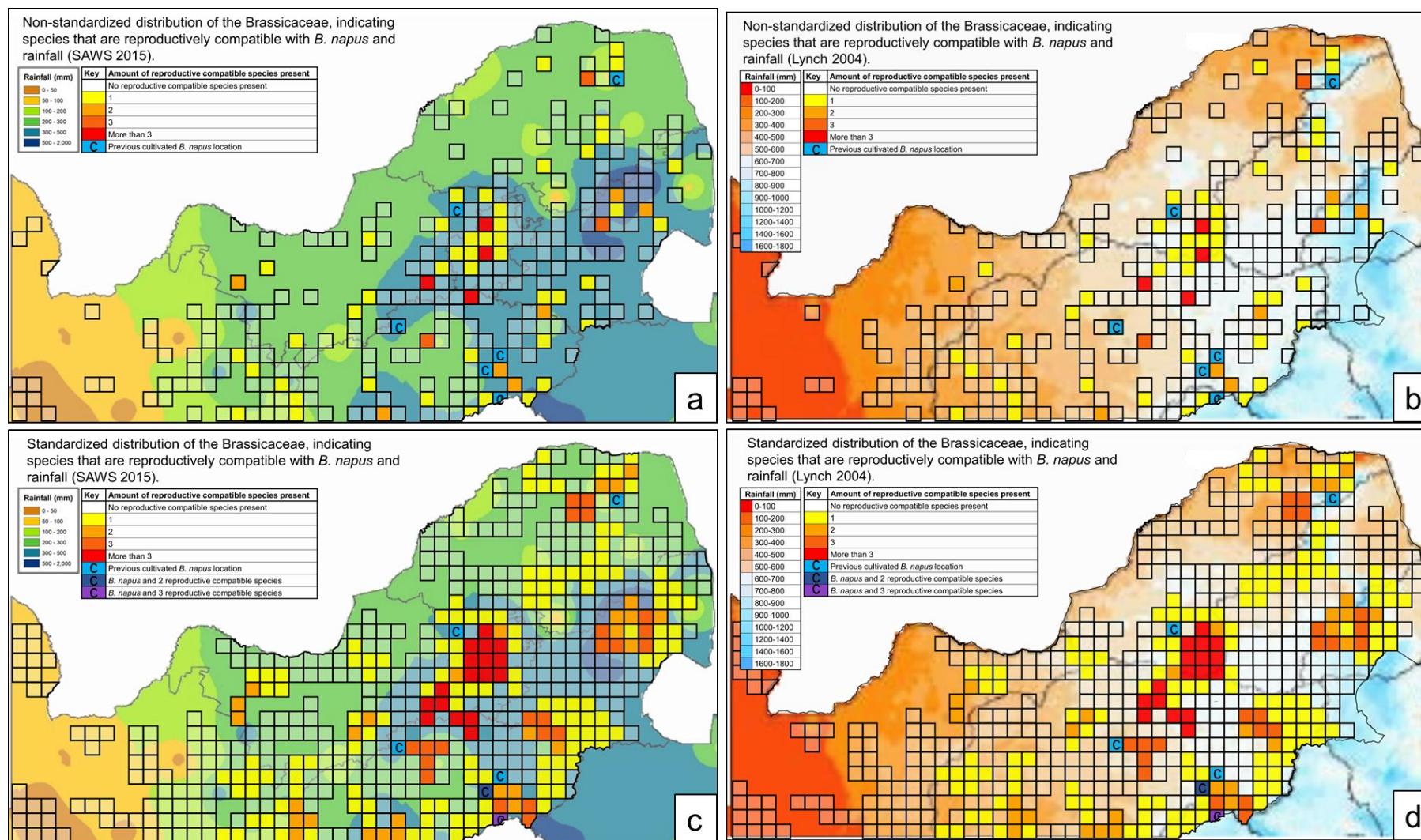


Figure 4.40: Non-standardized (a, b) and standardized (c, d) distribution of the Brassicaceae, indicating species that are reproductively compatible with *B. napus* in seasonal rainfall between July 2014 and January 2015 (SAWS 2015) (a,c) and mean rainfall between 1950 and 1990 (Lynch 2004) (b,d).

4.3.7 Brassicaceae phytochoria

The standardized species Brassicaceae distribution was selected to further determine the Brassicaceae phytochoria. The distribution was divided into floristic areas based on the presence of dominant Brassicaceae species and reproductively compatible diagnostic species (phytochoria), and their prevalence according to bioregions and rainfall. Five of the 12 standardized species clusters contains species that are reproductively compatible with *B. napus* (Table 4.5). Clusters two, three, four, seven, eight, nine, 11 and the outliers were coloured transparent to visually facilitate the selected regions.

These five clusters, as a representation of the Brassicaceae as a whole, were split into two separate groups. This was done according to rainfall with the vertical blue line separating overall low rainfall (left) and overall higher rainfall (right) (rainfall as in Figure 4.17) (Figure 4.41). The remaining three clusters containing reproductively compatible relatives each occur in mountainous areas and were split into three separate clusters (dark green, bright green and yellow circles (Figure 4.41)) according to the mountain that is present. Cluster one is present in the Soutpansberg area, one half of cluster 5 (central) on the eastern side of the Waterberg mountain range, and its second half (East) and cluster six in foothills of the Drakensberg mountain range.

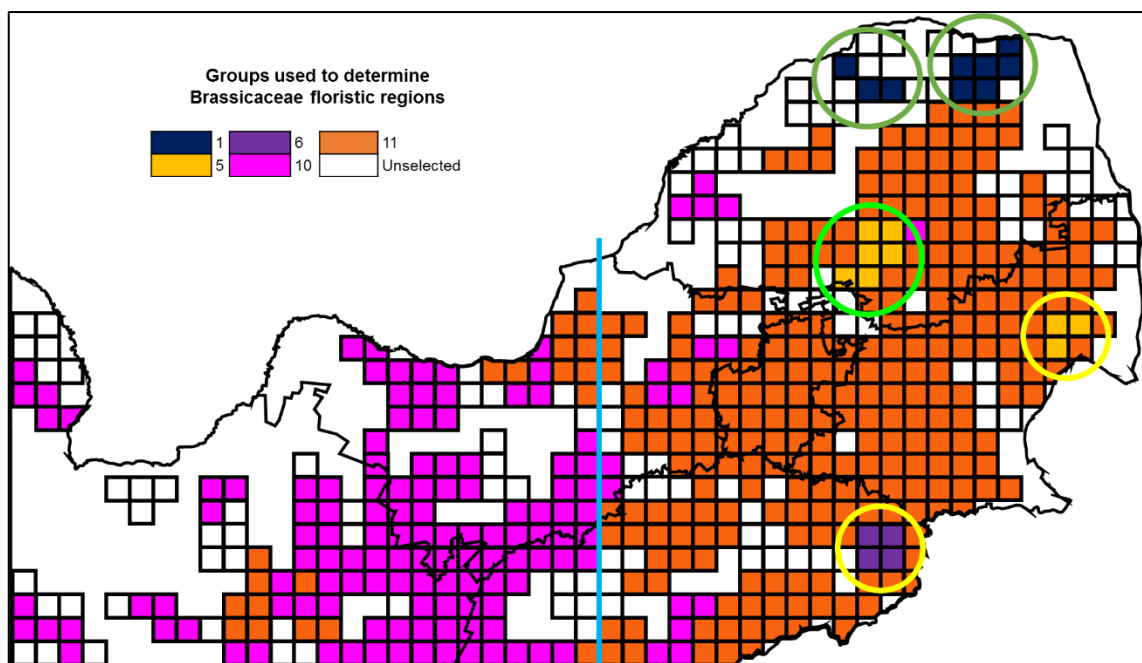


Figure 4.41: Phytochoria of the Brassicaceae. Cluster numbers are as indicated in Figure 4.15.

4.4 Discussion

4.4.1 Specimen data

Species distributions for this study were dependent on the available material from herbaria. These collection records are subjective to the places where they were collected, peaking in populated areas, with few to no specimens collected in areas where people rarely tread. Existing records were additionally subjected to curation policies of separate herbaria throughout the years of collection (throughout 1830 – 2013) with many specimens being donated to non-target herbaria.

To best determine the distribution of the Brassicaceae throughout the study area, specimen data was used due to the difficulties presented by conducting fieldwork within such a vast area. The Brassicaceae distributions available from herbaria have limited coordinate data so that QDGC's were used. The genera that were the most abundant according to non-standardized specimen data was visually represented in figure 4.3. *Brassica* (a), *Capsella* (b), *Coronopus* (d), *Diplotaxis* (e), *Erucastrum* (g), *Heliophila* (h), *Lepidium* (i), *Raphanus* (l), *Rorippa* (n) and *Sisymbrium* (p) and is distributed across the study area, with the most specimens collected in Gauteng (Appendix C; Figure 4.3).

Due to the use of specimen distributions and consequent sampling bias around cities, multiple specimens of a single species per QDGC were discarded to obtain presence/absence data. Converting the data to presence/absence data may no longer indicate the abundance of each species according to specimen data but rather indicates the distribution of species across the study area irrespective of the number of specimens collected, thus reducing the sampling bias. This data indicated *Sisymbrium* to be the dominant genus (occurring the most) in Gauteng, North-West and Northern Cape, *Heliophila* and *Lepidium* in Limpopo, and *Lepidium* in Free State and Mpumalanga (Table 4.2). Seventeen percent of the total species are *Lepidium* species, and the Free State contains 20% of the species within the study area (Table 4.2) according to specimen data standardized to presence/absence data. The Brassicaceae tribe is dominant in all provinces, except the Northern Cape where the Lepidieae dominate (27%) (Table 4.3).

Very few QDGC's were sampled so that the data was sparse and would limit the spatial assessment with regards to data of species within all the QDGC's present within the study area. To increase the number of QDGC's used, species were standardized using the Centroid Grid profile (see Chapter 3). The Centroid Grid profile considers surrounding QDGC's with the most similar vegetation to increase the number of species for the centroid grid. The QDGC's that

contain species data was increased by 295 with application of the Centroid Grid profile (Figure 4.4). The Centroid Grid profile increased the number of QDGC's used within each province of the study area, with the exception of Gauteng (Figure 4.5) where the low number of total QDGC's, small surface area (Figure 4.6) and high population density (Appendix C; Figure 4.7) most likely allowed the Gauteng Brassicaceae species to be collected more abundantly. Where there is high potential for collection due to high population densities, the number of species did not increase significantly (Figure 4.7). The Centroid Grid profile increased the number of species per QDGC and QDGC's containing Brassicaceae species in especially larger (Figure 4.5, 4.6) and less populated (Figure 4.7) provinces.

4.4.2 *Brassica napus* cultivation

Brassica napus was not cultivated in the northern provinces of South Africa (SAGIS 2015) during the time of study. This was ascribed to the high levels of irrigation required in the summer rainfall regions (DAFF 2010) and transport of the product to the grain silos present in the Western Cape (SOILL 2015) may be too expensive (fuel used relative to distance). The distribution of relatives that are reproductively compatible with *B. napus* overlaps with the areas where *Brassica* species have previously been cultivated in the study area (Figure 4.38). The areas of previous cultivated *B. napus* is important as it is the areas where the crop is most likely to be planted again in the future, and will be used during the risk assessment (Chapter 5), as well as to provide accurate recommendations (Chapter 6).

4.4.3 Similarity between clusters

The six data sets were defined with the initial objectives to establish phytochoria for the Brassicaceae (phytochoria) and to test whether the standardized data set improved the application of the specimen data. Each of the six data sets were inspected and compared to the remaining five data sets. Initial NMDS analyses, with Bray-Curtis similarity applied, indicated no groups that could be used for the establishment of phytochoria in any of the six data sets (Figures 4.8 (non-standardized species), 4.12 (standardized species), 4.18 (non-standardized genera), 4.22 (standardized genera), 4.28 (non-standardized tribes) and 4.32 (standardized tribes) respectively). Dendrograms were derived to obtain clusters with the use of SIMPROF tests and resemblance slices (Figures 4.9, 4.13, 4.19, 4.24, 4.30 and 4.33 respectively). Phytochoria emerged with the insertion of these clusters into NMDS (Figures 4.10, 4.14, 4.20, 4.23, 4.29 and 4.34 respectively). The clusters in each of the six data sets overlap one another considerably indicating that each of the clusters are similar to one another and may demonstrate the Brassicaceae not to be very dissimilar or diverse in species distribution across the study area.

The standardized clusters of each hierarchy were overall better indicators of the distribution of the Brassicaceae throughout the study area as the phytochoria of each standardized set (Figures 4.14 (species), 4.23 (genera) and 4.34 (tribes)) was better defined than its non-standardized (Figures 4.10 (species), 4.20 (genera) and 4.29 (tribes)) counterpart. This was further visually indicated by colouring QDGC's according to the phytochoria clusters in each data set (Figures 4.11, 4.15, 4.21, 4.25, 4.31 and 4.35 respectively).

These data sets were further assessed with the aid of ANOSIM and SIMPER to determine whether the Centroid Grid profile has enhanced the outcomes of the limited specimen data. These analyses indicated that there are relatively low similarities between the species present in every QDGC and every other QDGC in each of the six data sets (Tables 4.12, 4.13). The most similarities (ANOSIM) and least dissimilarities (SIMPER) occurred in the standardized tribe distribution of the Brassicaceae across the study area. This is as expected due to these species being combined into more generalized tribe clusters after being standardized by the Centroid Grid profile. No reproductively compatible relatives (Table 4.13) contributed to the dissimilarity between the non-standardized and standardized species or genera distributions (Table 4.12). The Brassicaceae as a whole, however, contributed the second most to the dissimilarity present within the non-standardized (22.06%) and standardized (23.34%) Brassicaceae tribe distributions (Table 4.12). These percentages may seem high but when considering that there are only ten Brassicaceae tribes present throughout the study area, these "high percentages" are not significant ($100\%/10 \text{ tribes} = 10\% \text{ per tribe}$).

Reproductively compatible relatives were dominant within at least one cluster in each of the six data sets (Tables 4.4–4.9). Even though these relatives are dominant in more than 75% of QDGC's in their respective clusters in the non-standardized data sets, only genera cluster (Cluster 4) and tribe cluster (Cluster 5) was deemed adequate (by considering number of QDGC's, species and individuals, mean, standard deviation and upper quartile (4.3.6)) to indicate the distribution of the reproductively compatible Brassicaceae species. Three of the five standardized clusters containing reproductively compatible relatives were deemed adequate (Clusters 1, 5 and 6) for indication of the distribution of reproductively compatible Brassicaceae relatives along with all four clusters containing reproductively compatible relatives of the standardized genera clusters (Clusters 1, 4, 6 and 9) and all three of the standardized tribe clusters (Clusters 6, 7 and 9).

The dominant taxa (diagnostic species) and taxa restricted to the cluster (characteristic species) were identified for each cluster within each data set (Tables 4.4, 4.5, 4.6, 4.7, 4.8 and 4.9 respectively). In each of the non-standardized data sets the reproductively compatible relatives

were distributed throughout multiple clusters: 7 out of 9 species clusters contained reproductively compatible relatives, 8 out of 8 genera clusters and 4 out of 8 tribe clusters. The standardized clusters were similarly compared to whether reproductively compatible relatives are present within and were identified as present in 7 out of the 12 species clusters, 8 out of the 9 genera clusters and 6 out of the 9 tribe clusters. The distribution of the reproductively compatible relatives is thus wide and indefinable.

The dominant species of the species distributions roughly coincide with the dominant genus and tribe distributions. As example, cluster four of the non-standardized distribution of the Brassicaceae genera identified *Brassica* as the dominant genus (Table 4.6). The area that this cluster occurs in is roughly similar to cluster 9 of the standardized genera distribution (Figure 4.21 compared to 4.25) that identified *Brassica* as the dominant genus as well (Table 4.7). The majority of these two clusters' QDGC's are within the Central Bushveld and Lowveld bioregions (Figure 4.27) (see 4.4.4).

4.4.4 Bioregions and rainfall

The distribution of the Brassicaceae is dependent upon a variety of multiple environmental factors including the geology of the area, precipitation and the means for distribution (mostly anthropological) (Mummenhoff *et al.* 2005; Pessel *et al.* 2001). Due to the standardized cluster distributions (determined by Bray-Curtis similarity dendrograms) of each of the standardized data sets presenting improved distributions of the Brassicaceae across the study area, these data sets (Figures 4.15, 4.25 and 4.35 respectively) were used for further analyses. To identify further factors that influence the distribution of the Brassicaceae, the standardized distribution of species, genera and tribe clusters were consequently overlapped with different maps for comparison.

Bioregions were obtained from Mucina & Rutherford (2006) and included a total of 35 bioregions for the whole of South Africa. Only eight different bioregions are present in the study area (see Chapter 3.1). Bioregions were overlaid onto standardized species, genera and tribe cluster distributions (Figures 4.16, 4.27 and 4.39 respectively). A recent seasonal rainfall chart was obtained from the South African Weather Service (SAWS 2015). It indicates the amount of rainfall, measured in mm, for the season of July 2014 to January 2015. This season was chosen due to *B. napus* being a winter crop (Jansen van Rensburg *et al.* 2007), and winter being from June to August. Another, long term rainfall chart constructed by Lynch (2004) indicating mean annual rainfall for 50 years (1950–1999), was chosen to indicate rainfall over time – an indication of rainfall during the time period wherein specimens used for data analyses may have

been collected. Standardized species, genera and tribe cluster distributions were overlaid onto seasonal and long-term rainfall (Figures 4.17, 4.26 and 4.40 respectively).

Rainfall generally correlates with bioregions. This is evident when comparing the rainfall chart to the bioregion map (see Chapter 3). Where the rainfall is up to 50 mm (during the season selected), the region is identified as the Bushmanland region. Between 50 and 100 mm is the Kalahari Duneveld region, 100 to 300 mm the Eastern Kalahari Bushveld bioregion and from 300 up to 500 mm is the Mesic Highveld Grassland bioregion (Figure 3.15 compared to Figures 3.17, 3.18). Higher rainfall occurs around mountain ranges due to orographic precipitation (AMS 2012).

When comparing the distribution of where *B. napus* was previously cultivated to rainfall areas on the rainfall chart (Figure 4.40), *B. napus* is present in regions where the seasonal rainfall is between 300 and 500 mm. Non-standardized species that are reproductively compatible with *B. napus* occurred most frequently in areas where the rainfall was between 300 and 500 mm, followed by 200–300 mm, with only two grids occurring where the season rainfall was 100–200 mm (Figure 4.40a,b). This tendency is repeated with the standardized species (Figure 4.40c,d) with the fewest QDGC's being in areas where the seasonal rainfall is between 100 and 200 mm. No reproductively compatible relatives occur where the season rainfall is less than 100 mm, in both non-standardized and standardized distributions. It is evident that these species prefer environments where there is high (>300 mm) precipitation.

4.4.5 Reproductive compatibility

Brassica napus is known to exchange genes with its wild and weedy relatives to form fertile hybrids. The cultivation of transgenic *B. napus* in the northern provinces of South Africa may present difficulties by releasing problematic transgenes into the environment. For this to occur, then 1) reproductively compatible Brassicaceae species must be present within the study area, and 2) these species must grow in close proximity to where *B. napus* is cultivated.

There is no information available regarding the potential hybridization of *B. napus* with its indigenous relatives in South Africa (McGeoch *et al.* 2009). The potential hybridization between *B. napus* and its naturalised relatives is indicated in Table 4.13 as reported by various sources (indicated). All instances where *B. napus* successfully hybridized with any of its relatives are indicated in Table 4.13 separately to indicate which species occur within the study area. Previous studies have indicated that volunteer *B. napus* and *B. rapa* may act as genetic bridges to transgenes from cultivated *B. napus* to additional wild and weedy relatives (Hall *et al.* 2000; Reagon & Snow 2006; Warwick *et al.* 2008). One additional reproductively compatible relative which may use *B. rapa* as a genetic bridge species within the study area was added, namely

Raphanus sativus. Instances where *B. juncea* hybridized with *B. napus* occurred the second most frequent and was included as an additional genetic bridge species, but no additional new reproductively compatible relatives were added. All identified reproductively compatible species are present within the Brassiceae (tribe) (Table 4.13; Appendix B).

The distribution of the reproductively compatible relatives were plotted to indicate the number of these species present per QDGC, as well as an indication of areas where canola had previously been cultivated (Figure 4.38). Standardization of data via the Centroid Grid profile increased the distribution (number of QDGC's) and number of total reproductively compatible relatives within the study area (Figure 4.38). These figures were further overlapped with bioregions (Mucina & Rutherford 2006) (Figure 39) and seasonal (SAWS 2015) (Figure 4.40a,c) and long-term rainfall (Lynch 2004) (Figures 4.40b,d).

The reproductively compatible species of *B. napus* occurs mostly in the Grassland Biome, along the border with KwaZulu-Natal, and in the Northernmost parts of Limpopo (Figure 4.39). The most reproductively compatible relatives occurred in the Mesic Highveld Grassland and Dry Highveld Grassland Bioregions (Figure 4.39). The most reproductively compatible relatives occur where rainfall is highest with no reproductively compatible relatives in most of the Northern Cape due to low rainfall (Figure 4.40).

4.4.6 Brassicaceae phytochoria

The standardized species data set (4.3.2.2) was selected to determine phytochoria from its phytochoria clusters (Figures 4.13–4.15). The clusters that contained diagnostic and dominant reproductively compatible relatives (Tables 4.5 and 4.13) were isolated to determine phytochoria (Figure 4.42). The Brassicaceae were divided according to rainfall (1) and according to cluster presence in mountainous areas (2) (Figure 4.41). Each of the mountain ranges were identified for its three phytochoria (2A–2C) (Figure 4.41).

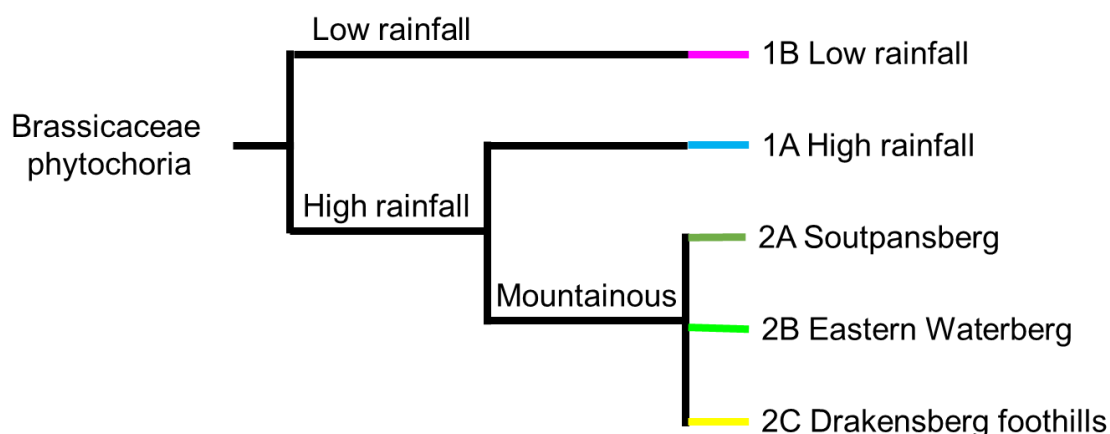


Figure 4.42: Division of the Brassicaceae phytochoria. Colours of phytochoria as indicated in Figure 4.41.

The dominant species within the high rainfall phytochorion (1A) are *Capsella bursa-pastoris*, *Coronopus integrifolius*, *Erucastrum griquense*, *Erucastrum strigosum*, *Heliophila deserticola*, *Heliophila minima*, *Heliophila suavissima*, *Lepidium africanum*, *Matthiola torulosa*, *Rapistrum rugosum*, *Rorippa fluviatilis* var. *caledonica*, *Sisymbrium burchellii* var. *burchellii*, and *Sisymbrium capense* (standardized species clusters three, four and ten) (Table 4.5). *Brassica rapa*, *Capsella bursa-pastoris*, *Cardamine africana*, *Diplotaxis muralis*, *Erucastrum austroafricanum*, *Erucastrum strigosum*, *Heliophila carnosa*, *Heliophila rigidiuscula*, *Heliophila* species, *Lepidium africanum*, *Lepidium bonariense*, *Lepidium* species, *Lepidium schinzii*, *Lepidium transvaalense*, *Lepidium virginicum*, *Nasturtium officinale*, *Raphanus raphanistrum*, *Rorippa fluviatilis* var. *fluviatilis*, *Rorippa nudiuscula*, *Sisymbrium* species, *Sisymbrium thellungi* and *Sisymbrium turczaninowii* (clusters two, seven, eight and 12) are dominant within the low rainfall phytochorion (1B) (Table 4.5). Within the mountainous regions *Brassica rapa* is dominant (cluster one) within the Soutpansberg phytochorion (2A), *Sinapis arvensis* (cluster five) within the Eastern Waterberg phytochorion (1B), and *Lepidium transvaalense*, *Raphanus raphanistrum* and *Sinapis arvensis* (clusters five and six) within the Drakensberg foothills phytochorion (2C) (Table 4.5).

When looking specifically at dominant reproductively compatible species *Rapistrum rugosum* dominates the low rainfall (1A), *Brassica rapa*, *Diplotaxis muralis* and *Raphanus raphanistrum* the high rainfall (1B), *Brassica rapa* in the Soutpansberg (2A), *Sinapis arvensis* in the Eastern Waterberg (2B) and *Raphanus raphanistrum* and *Sinapis arvensis* in the Drakensberg foothills Brassicaceae phytochoria (2C) (Table 4.5).

The high rainfall Brassicaceae phytochorion comprises of the largest surface area (311 QDGC's) followed by the low rainfall (162 QDGC's) and Soutpansberg (9 QDGC's)

Brassicaceae phytochoria (Figures 4.15, 4.41; Table 4.5). The Eastern Waterberg and Drakensberg foothills Brassicaceae phytochorion each consist of six QDGC's (Figures 4.15, 4.41; Table 4.5). Standardized species clusters nine and 11 were not assigned to a specific region due to their distribution occurring throughout multiple of the regions.

4.5 Summary

The diversity of all wild and weedy Brassicaceae relatives of *Brassica napus* was quantified and were widespread across northern South Africa. The most species, according to specimen data, were present in the areas with the highest anthropological population densities due to sampling bias. To remedy this phenomenon to a degree, specimen data was standardized to include only one individual per species per QDGC in the study area for further spatial analysis.

The spatial overlay of the Brassicaceae was assessed as a basis for understanding the potential for gene flow to occur from commercially-produced canola to its reproductively compatible relatives. The spatial assessment of the Brassicaceae has indicated that several taxa occur in the same proximity that *B. napus* was previously cultivated in, albeit no reproductively compatible relatives occur in the same QDGC's. However, when standardizing the data *Diploaxis muralis*, *Raphanus raphanistrum* and *Rapistrum rugosum* (known to be reproductively compatible with *B. napus*) is present in the same QDGC's that *B. napus* have previously been cultivated in. These reproductively compatible relatives are the most likely to overcome the spatial barrier presented by gene flow.

Standardization by vegetation unit (Mucina & Rutherford 2006) using the Centroid Grid profile allows the most likely distribution of the Brassicaceae to be determined relatively accurately. An additional 295 QDGC's contain species of the Brassicaceae when the data is standardized. Bioregions (Mucina & Rutherford 2006) and rainfall (Lynch 2004; SAWS 2015) were selected as possible environmental factors that contribute to the distribution of the Brassicaceae. Bioregions and rainfall are correlated and the non-standardized to standardized Brassicaceae are distributed accordingly. The Brassicaceae occurs in areas where rainfall is highest, throughout the Mesic Highveld Grassland and Dry Highveld Grassland bioregions. The species that are reproductively compatible with *B. napus* consequently predominates in high rainfall areas – throughout the Mpumalanga and half of the Free State provinces (Mesic Highveld Grassland bioregion) and in the region from Gauteng to Kimberley where the sampling bias is most prominent.

The phytochoria for the Brassicaceae have shown that standardization via the Centroid Grid profile has improved the outcome. The 1) presence of reproductively compatible Brassicaceae

relatives in the northern provinces (4.4.1), and 2) whether these relatives grow in close proximity to where canola is cultivated (4.4.3) is considered.

These reproductively compatible species are considered and assessed whether they are associated with any specific phytochoria (4.4.2), phytochorion (4.4.4), bioregions or rainfall isohets (4.4.5). The aim is to use these categories as markers to define areas where transgenic *B. napus* cannot be cultivated in the future. We then also consider areas where non-transgenic canola have been cultivated in the past to determine whether reproductively compatible species occur in the same areas. If so, these cultivation zones can be flagged as problem areas.

Little to no studies have been done on the reproductive compatibility between *B. napus* and its relatives indigenous to South Africa. Future studies should remedy this, as it will serve to improve future spatial risk assessments of *B. napus* in South Africa.

4.6 References

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Chapter 5: Spatial Risk Assessment of *Brassica napus*

5.1 Introduction

Brassica napus L. is a cosmopolitan crop that belongs to the Brassicaceae Burnett, *nom. cons.* (Cruciferae Jussieu, *nom. cons. et nom. alt.*) – a family that is known for their ability to form fertile hybrids when gene flow occurs between related relatives (Al-Shehbaz *et al.* 2006; APG III 2009; Gueritaine *et al.* 2002; Warwick *et al.* 2003). *Brassica napus* is an amphiploid (taxon containing a diploid set of chromosomes derived from each parent species) oilseed crop hybrid between *B. rapa* L. and *B. oleracea* L. (Allender & King 2010; Simard *et al.* 2002; Warwick *et al.* 2010). There are numerous *B. napus* lineages, resulting in multiple crop varieties (Rieger *et al.* 2002; Song & Osborn 1992).

One of the most important components of a risk assessment is to evaluate the probability of gene flow from transgenic crops to their wild and weedy relatives. A risk assessment is a requirement to identify possible threats to conservation and ecological risks posed by genetically modified organisms (Hails & Morley 2005; McGeoch *et al.* 2009). Members of the Brassicaceae are known to hybridize readily, including cultivated species (*Brassica napus* and *B. rapa*), to form fertile hybrids (Al-Shehbaz *et al.* 2006; APG III 2009; Ellstrand & Schierenbeck 2000; Gueritaine *et al.* 2002; Warwick *et al.* 2003). *Brassica napus*, specifically, is known to hybridize with several of its wild and weedy relatives (Allainguillaume *et al.* 2006; Allender & King 2010; Anjum *et al.* 2012; Aono *et al.* 2006; FitzJohn *et al.* 2007; Scheffler & Dale 1994; Warwick *et al.* 2003). It overcomes reproductive barriers to form fertile hybrids with relatives such as *B. rapa*, *Erucastrum gallicum* and *Raphanus raphanistrum* (Légère 2005; McGeoch *et al.* 2009; Stewart *et al.* 2003; Warwick *et al.* 2003). These abilities raise the concern that seed and/or pollen from the transgenic *B. napus* will move from the site of cultivation and contaminate non-transgenic wild and weedy relatives resulting in transgenic hybrid species which is considered to be biological invasion at gene, species and ecosystem levels (Petit 2004; Vila *et al.* 2000).

Brassica napus is 20–40% allogamous (see Appendix A) and non-transgenic varieties have been improved through traditional cross-breeding with its wild relatives to enhance beneficial traits such as heat tolerance (Grubben & Denton 2004; Senior & Dale 2002). With its ability to form hybrids with many of its relatives, it is thought that non-transgenic *B. napus* will only remain uncontaminated under conditions where it is grown in isolated areas (Hall *et al.* 2000). *Brassica napus* also possesses the potential for secondary seed dormancy (D’Hertefeldt *et al.*

2008; Gueritaine *et al.* 2002; Warwick *et al.* 2003). Seed of this species may remain in the seedbank for up to ten years and may therefore commonly produce volunteer plants which could include hybrids (D'Hertefeldt *et al.* 2008; Warwick *et al.* 2003).

Brassica napus has been genetically modified (GM) to contain transgenes encoding for herbicide tolerance, insect resistance, and tolerance to other biotic stresses (see Chapter 2.6; Appendix A). GM herbicide tolerant *B. napus* was cultivated in Canada during 1982 for the first time (Senior & Dale 2002), and since was first commercialized during 1995 (Simard *et al.* 2002). Transgenic varieties of *B. napus* have been commercially planted in thirteen countries throughout the world, including Australia, Canada, China, Japan, and the United States of America (ISAAA 2014) (see Chapter 2).

Should transgenic varieties of *B. napus* cross with non-transgenic varieties or wild relatives, then this introduction of foreign genes through hybridization may be seen as biological invasion at either genetic or ecological levels (Petit 2004; Vila *et al.* 2000). Genetically, hybridization with *B. napus* affects the F₁ hybrid genepool by transferring transgenes from *B. napus* to its wild and weedy relatives (Vila *et al.* 2000). These transgenes may be beneficial to the hybrid species, allowing an increase in the genetic diversity, and the colonization and dispersal ability of the hybrid (Barbour *et al.* 2010; Hauser *et al.* 1998; Stewart *et al.* 2003; Vila *et al.* 2000). Alternatively, wild and weedy relatives may be threatened due to outbreeding depression or the dilution of the natural gene pool (Hails & Morley 2005; Vila *et al.* 2000). Ecologically, the hybridized species with an improved genepool might become more weedy and competitive under certain selection pressures, which in turn may threaten the natural species by competing for the same resources when present in the same environment (Halfhill *et al.* 2002; Vila *et al.* 2000).

The aim of the spatial risk assessment was to assess the probability that gene flow may occur from *B. napus* at the site of cultivation to contaminate reproductively compatible wild and weedy relatives. The risk assessment will consider the prevalence, spatial overlap, gene flow rate and distribution networks of *B. napus*. Further focus is placed on identifying possible implications to gene flow should transgenic *B. napus* be approved for cultivation in the northern provinces of South Africa.

5.2 Materials and methods

5.2.1 Data collection and processing methods

A literature study was conducted to determine which species of the Brassicaceae occurred in the study area. *All data was collected and processed as described in Chapters 3 and 4.*

Specimen data (specifically the location data) were collected from various major herbaria (12 in total) throughout the study area (Gauteng, Limpopo, Mpumalanga, North-West, Free State and Northern Cape). The QDGC data was further standardized using the Centroid Grid profile (Kurzweg 2011) and vegetation units as described by Mucina & Rutherford (2006) in order to extrapolate the extent of occurrence of the Brassicaceae. The QDGC data was refined and processed and subjected to similarity Bray-Curtis in order to obtain clusters by means of dendrograms (see Chapter 4).

A literature study identified Brassicaceae relatives which were reproductively compatible with *B. napus* and these were checked with the QDGC data to determine which were present in the study area (Chapter 4: Table 4.13). The distribution of these reproductively compatible relatives per QDGC, both non-standardized and standardized, were mapped and stacked on bioregions of South Africa as described by Mucina & Rutherford (2006), seasonal rainfall obtained from the South African weather service (SAWS 2015), and previous cultivation areas of non-transgenic *B. napus*, to determine areas of high likelihood for gene flow.

5.2.2 Spatial risk assessment method

The data obtained from the spatial assessment was used to rank each of the Brassicaceae species according to the likelihood for gene flow to occur between *B. napus* and its reproductively compatible relatives. This qualitative spatial risk assessment of reproductively compatible relatives considered the following factors (McGeoch 2009): (1) higher prevalence of any given species presents a higher chance for potential gene flow to occur; (2) spatial overlap between *B. napus* and its relatives increase likelihood for gene flow; (3) higher gene flow rate increases the potential for gene flow to occur; and (4) the presence of anthropological distribution networks (roads and railway lines) through areas where reproductively compatible relatives are present increases the potential for these relatives to distribute and cross spatial barriers.

5.2.2.1 Prevalence

The rank used for prevalence was determined by using both specimen data (the number of specimens collected per species) and distribution data (non-standardized and standardized) (Appendix C). Specimen data per species is as indicated in the species list (Appendix B). The distribution data was standardized so that a single QDGC contained only one individual per species; presence/absence data (Appendix C). Standardized occurrences (S) (maximum distribution: number of QDGC's covered) (Appendix C) was divided by the non-standardized occurrences (presence/absence distribution as per specimen data: number of QDGC's covered) (Appendix B) to obtain the proportional increase in distribution as determined by the Centroid Grid profile. The number of specimens collected (Sd) was multiplied by the proportional increase in distribution to obtain the estimated prevalence per reproductively compatible relative species. The estimated prevalence per reproductively compatible relative sorted from highest to lowest and used to rank each species, with a 1 indicating a species presenting the highest prevalence – this is then assumed also the highest likelihood for gene flow to occur.

Relative prevalence/species = $Sd(S_{max}/U)$, with S, standardized occurrences; U, non-standardized occurrences; Sd, number of specimens.

5.2.2.2 Spatial overlap

QDGC's where *B. napus* had previously been cultivated and surrounding grids (Chapter 4) were identified to determine the ranking for each reproductively compatible relative as an indication of spatial overlap (Appendix C). Reproductively compatible relatives recorded from the same QDGC's (h) as where *B. napus* had previously been cultivated were deemed of more importance than those present in the surrounding QDGC's (g) and were multiplied by two to increase their significance. The QDGC's containing reproductively compatible relatives in the same and surrounding QDGC's where *B. napus* had previously been cultivated were counted and added together for both the non-standardized and standardized distributions. The sum obtained was sorted from highest to lowest and used to rank each reproductively compatible species according to prevalence, with 1 indicating the most spatial overlap – this is then assumed also the highest likelihood for gene flow to occur.

Spatial overlap/species = $2\sum h + \sum g$, where h, QDGC shared by *B. napus* and reproductively compatible relative; g, QDGC with reproductively compatible relative bordering *B. napus* QDGC.

5.2.2.3 Gene flow rate

FitzJohn *et al.* (2007) identified the number of reported hybridization successes and failures for several of the Brassicaceae relatives, divided to indicate female and male results. The female and male results were considered together to obtain the total hybridization successes and failures as reported between *B. napus* and each of the reproductively compatible relatives present within the study area. The gene flow rate of one of the confirmed reproductively compatible relatives, *Eruca sativa* (Fahleson *et al.* 1997), was not determined by FitzJohn *et al.* (2007).

The number of successes (s) was divided by the number of failures (f) to obtain the proportional hybridization rate per reproductively compatible species. The sum obtained was sorted from highest to lowest and used to rank each reproductively compatible species according to gene flow, with 1 indicating the highest gene flow rate – this is then assumed also the highest likelihood for gene flow to occur.

Gene flow rate/species = s/f , with f, number of reproductive failures; s, number of reproductive successes.

5.2.2.4 Distribution networks

The Brassicaceae is known to be distributed by anthropological activities (Barnaud *et al.* 2013; Crawley & Brown 1995; Pessel *et al.* 2001; Schoenenberger & D'Andrea 2012). As such, the number of QDGC's that contain railway lines (rl) and reproductively compatible relatives were counted, followed by the number of QDGC's containing reproductively compatible relatives that surround (Appendix C) a QDGC where a railway line is present. The same procedure was repeated with QDGC's containing main roads (mr). The sum of these occurrences per species was sorted from highest to lowest and used to rank each reproductively compatible species according to distribution network intensity, with a 1 indicating the most distribution networks and it is then assumed also the highest likelihood for gene flow.

Distribution networks/species = $\sum rl + \sum mr$, with rl, QDGC containing a railway line; mr, QDGC containing a main road.

5.2.3 Risk assessment maps

Maps of both the non-standardized and standardized Brassicaceae distributions that indicate the presence of confirmed reproductively compatible relatives were produced. Further distribution maps of both the non-standardized and standardized data sets of reproductively

compatible relatives were produced to indicate the likelihood for gene flow to occur within each QDGC based on the spatial risk assessment. Risk values were calculated for each QDGC using the ranks assigned to each reproductively compatible species as determined by the four factors that support gene flow.

The sum total of these factors (prevalence, spatial overlap, gene flow rate and the presence of anthropological distribution networks) was calculated (Σ) and used to assign ranks to each reproductively compatible relative as an indicator of its total potential for gene flow to occur. The species that obtained the lowest sum total was assigned the highest rank indicating the species presenting the highest likelihood for gene flow to occur. The species presenting the highest potential gene flow will be a species with a high relative prevalence, known to hybridize with *B. napus*, of which the area of distribution overlaps with that of *B. napus* and that has the potential to distribute to further relatives by means of anthropological interventions.

Each QDGC was analysed individually. The reproductively compatible relatives present in every QDGC (Appendix D) were identified and the numbers assigned to each of the ranked relatives were added together. These numbers were inverted for more accurate results, with the highest scores indicating species that present the highest risk. For example, if a specific QDGC contained relatives ranked second, fourth and fifth, the combined score for that QDGC would be nineteen ($8 + 6 + 5 = 19$). Inverting the scores prevent a QDGC with a single high ranked species from attaining a higher total rank than a QDGC containing two high ranked species because of a lower score. The QDGC that scored the highest therefore presents the highest potential likelihood for gene flow to occur. The scores obtained were divided into categories of equal size range and coloured accordingly when producing distribution maps (non-standardized and standardized) that indicate the potential risk for hybridization per QDGC.

5.3 Results and Discussion

Data of 2296 Brassicaceae specimens were collected from herbaria, show 83 species to be present in the study area. The literature study identified ten Brassicaceae species to be reproductively compatible with *B. napus* and to be present in the study area (Chapter 4, Table 4.13). *Brassica rapa* was identified as the species that is best known to hybridize with *B. napus* (Allainguillaume *et al.* 2006; Allender & King 2010; Anjum *et al.* 2012; Aono *et al.* 2006; FitzJohn *et al.* 2007; Scheffler & Dale 1994; Warwick *et al.* 2003). According to FitzJohn *et al.* (2007), *B. rapa* has the highest median rate of hybrid production, followed by *B. juncea*, when only comparing species that are present in the study area. Assuming that gene flow occurs from *B. napus* to these relatives, *B. rapa* and *B. juncea* present an additional hypothetical risk by

possibly acting as genetic bridge species. When comparing the Brassicaceae relatives that are reproductively compatible with *B. juncea* and *B. rapa* as determined by FitzJohn *et al.* (2007), the only other species of importance in the study is *Raphanus sativus* (Chapter 4: Table 4.13). There is no information available regarding potential reproductive compatibility between *B. napus* and its indigenous relatives in South Africa (McGeoch *et al.* 2009).

Each of the species that were identified as reproductively compatible with *B. napus* (Chapter 4: Table 4.13) were compared using the four factors: prevalence, spatial overlap, gene flow rate and anthropological networks of distribution.

5.3.1 Prevalence

Relative prevalence was ranked per species, with the highest rank (1) allocated to the species of which the most specimens were collected and that occurred in the most QDGC's (largest distribution). *Raphanus raphanistrum* was ranked highest for prevalence in the study area (Table 5.1). It was collected 37 times and was distributed throughout 24 QDGC's in the study area (Appendix C). When the distribution of *R. raphanistrum* was standardized by Centroid Grid profile it occurred throughout 82 QDGC's (Appendix C), making it the reproductively compatible species with the widest estimated distribution. While *B. rapa* was collected 17 times throughout 14 QDGC's, its distribution expanded to 56 QDGC's when standardized by the Centroid Grid profile (Appendix C). The prevalence of the reproductively compatible relatives declined in the subsequent species with the least specimens belonging to *B. nigra* and *Sinapis alba* which was each collected only twice in two different QDGC's (Appendix C).

Table 5.1: Calculations of prevalence and ranking of species.

Reproductively Compatible Relative	Specimen data (Sd)	Non-standardized (U)	Standardized (S)	S/U	Sd (S/U)	Rank
<i>Brassica juncea</i>	5	5	20	4.0	20.0	6
<i>Brassica nigra</i>	2	2	8	4.0	8.0	9
<i>Brassica rapa</i>	17	14	56	4.0	68.0	3
<i>Diplotaxis muralis</i>	26	13	56	4.3	111.8	2
<i>Eruca sativa</i>	6	3	10	3.3	19.8	7
<i>Raphanus raphanistrum</i>	37	24	82	3.4	125.8	1
<i>Raphanus sativus</i>	3	2	8	4.0	12.0	8
<i>Rapistrum rugosum</i>	15	12	41	3.4	51.0	4
<i>Sinapis alba</i>	2	2	8	4.0	8.0	9
<i>Sinapis arvensis</i>	7	7	35	5.0	35.0	5

5.3.2 Spatial Overlap

Spatial overlap was determined by comparing the distribution of the confirmed reproductively compatible relatives to QDGC's in which *B. napus* had previously been cultivated (Chapter 4.3.6). Non-standardized and standardized distributions of QDGC's containing reproductively compatible species were counted when present in either the same QDGC where *B. napus* had previously been cultivated, or when it was present in the surrounding QDGC's as a means of determining the maximum potential distribution to which gene flow may occur with regards to spatial overlap.

The distribution of each of the reproductively compatible populations from the source (crop field) to the nearest reproductively compatible population, and the next, is an indication of the furthest reaches that a specific gene may distribute to introgress into each population. The further away a population occurs from the source of the gene (crop field), the less likely it will be for gene flow to occur (Quist & Chapela 2001; Rieger *et al.* 2002; Staniland *et al.* 2000). For this reason, reproductively compatible relatives present within the same QDGC where *B. napus* had previously been cultivated were considered to present the highest likelihood for gene flow to occur and scored higher (Table 5.2).

Table 5.2: Calculations of spatial overlap and ranking of species. U, non-standardized QDGC data; S, standardized QDGC data.

Reproductively Compatible Relative	Same QDGC U (h_1)	Same QDGC S (h_2)	Surrounding QDGC U (g_1)	Surrounding QDGC S (g_2)	$2(h_1 + h_2) + (g_1 + g_2)$	Rank
<i>Brassica juncea</i>	0	0	2	4	6	5
<i>Brassica nigra</i>	0	0	0	0	0	9
<i>Brassica rapa</i>	0	0	1	6	7	4
<i>Diplotaxis muralis</i>	0	1	2	4	8	3
<i>Eruca sativa</i>	0	0	0	1	1	8
<i>Raphanus raphanistrum</i>	0	2	2	5	11	1
<i>Raphanus sativus</i>	0	0	1	2	3	7
<i>Rapistrum rugosum</i>	0	1	3	6	11	1
<i>Sinapis alba</i>	0	0	0	0	0	9
<i>Sinapis arvensis</i>	0	0	0	5	5	6

Based on the non-standardized data, there were no species that occurred in the same QDGC's as old fields of *B. napus* (Appendix C). When looking at the QDGC's surrounding those that *B. napus* was cultivated in, *Rapistrum rugosum* occurred three times, followed by *B. juncea*, *D.*

muralis and *Raphanus raphanistrum* that occurred twice. The standardized distribution supplements *R. raphanistrum* in the same QDGC where a *B. napus* crop had previously been cultivated, followed by *B. rapa* and *Rapistrum rugosum* occurring in only one and two QDGC's respectively. The surrounding standardized QDGC's were dominated by *B. rapa* and *R. rugosum* with six each (Appendix C). According to collection data, *B. nigra* and *Sinapis alba* did not occur in either the same or QDGC's surrounding where *B. napus* had previously been cultivated.

5.3.3 Gene flow rate

The rate of gene flow between two populations is influenced by the distance between the populations, effect of frequency and distribution of volunteers, and time (Beckie *et al.* 2003). *Eruca sativa* is known to hybridize with *B. napus* (Fahleson *et al.* 1997), but the rate thereof was not determined by FitzJohn *et al.* (2007). For this reason, *E. sativa* was assigned a low ranking of 9 (Table 5.1).

Table 5.3: Calculations of gene flow rate based on reproductive success or failure of ranking species. s, success; f, failure.

Reproductively Compatible Relative	Success:Failure (FitzJohn <i>et al.</i> 2007)		Combined	s/f	Rank
	Male	Female			
<i>Brassica juncea</i>	25:1	13:4	38:5	7.6	2
<i>Brassica nigra</i>	2:2	4:2	6:4	1.5	4
<i>Brassica rapa</i>	55:8	84:0	139:8	17.375	1
<i>Diplotaxis muralis</i>	3:0	1:1	4:1	4	3
<i>Eruca sativa</i>	–	–	–	–	10
<i>Raphanus raphanistrum</i>	0:4	3:2	3:6	0.5	5
<i>Raphanus sativus</i>	1:5	1:2	2:7	0.283	7
<i>Rapistrum rugosum</i>	–	1:0	1:0	0	9
<i>Sinapis alba</i>	0:6	1:2	1:8	0.125	8
<i>Sinapis arvensis</i>	1:10	5:8	6:18	0.3	6

Unfortunately, data regarding gene flow likelihood between the indigenous Brassicaceae relatives in South Africa is lacking (McGeoch *et al.* 2009). FitzJohn *et al.* (2007) identified 139 reported cases of *B. napus* successfully hybridizing with *B. rapa* (Table 5.3). When calculating the proportion of successes to failures, *B. napus* and *B. rapa* will hybridize 17 times to one.

5.3.4 Anthropological distribution networks

The distribution of crop seeds, particularly the Brassicaceae, is largely due to anthropological activity (Barnaud *et al.* 2013; Crawley & Brown 1995; Reagon & Snow 2006). Crawley & Brown (1995) described *B. napus* to be common along main roads in the United Kingdom, with species being present for longer periods of time when the seed production was high. Furthermore, Schoenenberger & D'Andrea (2012) documented transgenic *B. napus* along railway lines in Switzerland during 2012 and Pessel *et al.* (2001) along road verges during 2000. In all probability, seed of *B. napus* is distributed as it is transported (Pessel *et al.* 2001; Schoenenberger & D'Andrea 2012).

The presence of railway lines and roads in the QDGC's and surrounding QDGC's where *B. napus* was previously cultivated would thus increase the chances for some of its confirmed reproductively compatible relatives to distribute, and gene flow to occur. As such, anthropological methods of distribution, or in this case, transportation, was included as a factor when ranking species according to their likelihood to hybridize with *B. napus*.

To illustrate anthropological activity present in the study area railway lines and main roads (chart obtained from UN (2007)) were stacked with the distribution of reproductively compatible relatives (Figure 5.1). These networks of transportation serve as potential seed distribution routes and were therefore included in the risk assessment rank table. For this purpose the QDGC's that contained reproductively compatible relatives and that were also crossed by either a road or railway line were identified. These QDGC's, as well as the number of QDGC's that contained reproductively compatible relatives bordering a QDGC that contained roads and railway lines, were then counted for further ranking (Table 5.4).

$$\sum rl + \sum mr$$

Table 5.4: Calculation of anthropognetic distribution networks based on the presence of transport routes and ranking of species.

Reproductively Compatible Relative	Railway line; Same QDGC (rl_1)	Railway line; Surrounding QDGC (rl_2)	Main Road; Same QDGC (mr_1)	Main Road; Surrounding QDGC (mr_2)	$(rl_1 + rl_2) + (mr_1 + mr_2)$	Rank
<i>Brassica juncea</i>	1	3	2	1	7	6
<i>Brassica nigra</i>	1	1	2	0	4	8
<i>Brassica rapa</i>	5	5	7	3	20	2
<i>Diplotaxis muralis</i>	7	2	6	3	18	3
<i>Eruca sativa</i>	3	0	2	0	5	7
<i>Raphanus raphanistrum</i>	13	6	13	8	40	1
<i>Raphanus sativus</i>	1	0	1	1	3	10
<i>Rapistrum rugosum</i>	7	2	6	3	18	3
<i>Sinapis alba</i>	1	1	1	1	4	8
<i>Sinapis arvensis</i>	4	3	4	2	13	5

It is evident that several reproductively compatible species have distributed along main roads and railway lines (Figure 5.1). One example of where anthropological influence may be observed is along the QDGC's in the southern-most parts of the study area, where a single railway line connects five different QDGC's that contain reproductively compatible relatives of *B. napus* (red circle on Figure 5.1a). Additionally, it is clear that the most reproductively compatible relatives occur in the vicinity of major cities or towns, and along railway lines and roads.

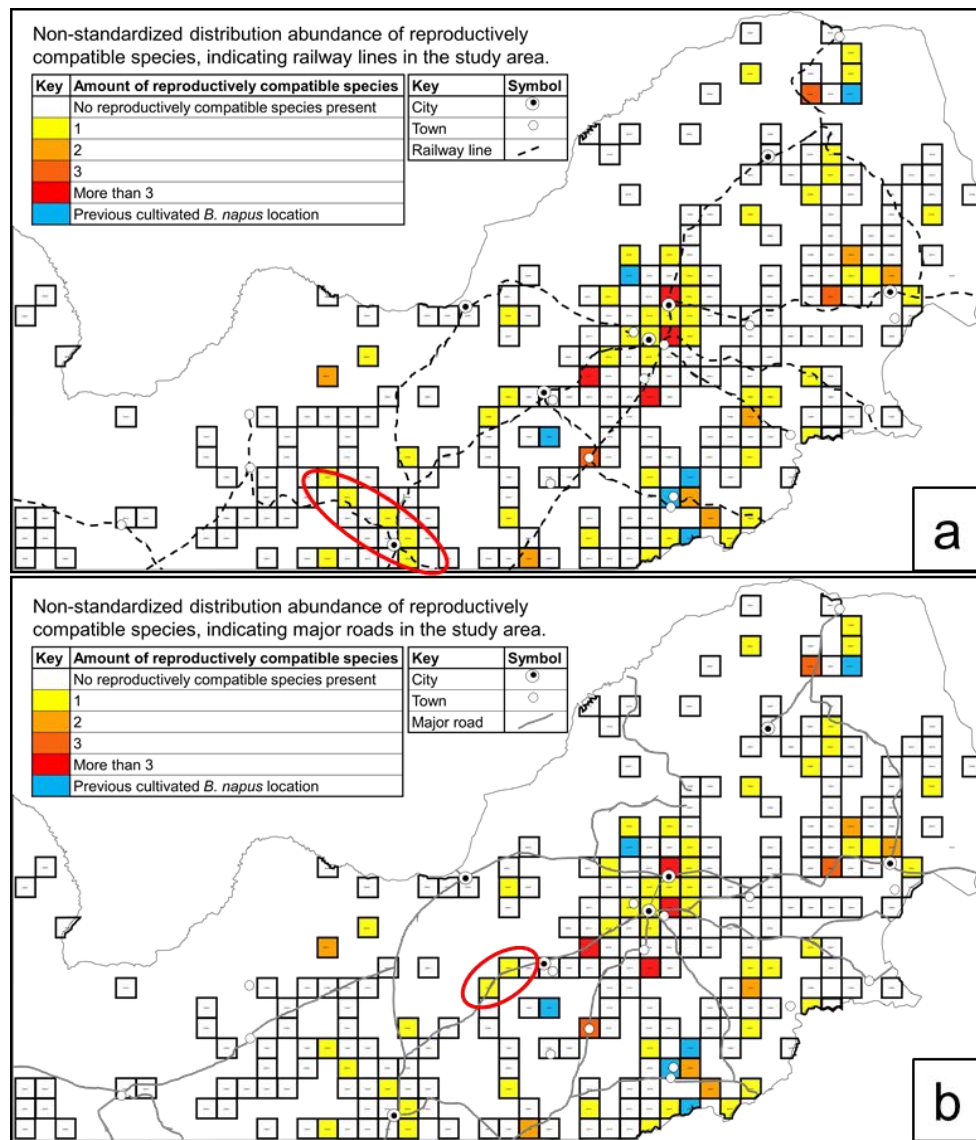


Figure 5.1: Cities, towns, railway lines (a) and roads (b) (obtained from UN (2007)) that overlapped with the distribution of Brassicaceae relatives that are reproductively compatible with *Brassica napus*, indicating number of species present in each QDGC. The red polygon indicates examples of areas where a species may have been anthropologically distributed along a railway line and road respectively.

5.3.5 Spatial risk assessment by species occurrences

5.3.5.1 Preliminary spatial risk assessment

The number of reproductively compatible relatives (Chapter 4: Table 4.13) per QDGC (Appendix C) is visually represented (Figure 5.2) suggesting that specimens were more frequently collected where there was higher potential for collection. This has been indicated and was also illustrated in the data and figures that were constructed.

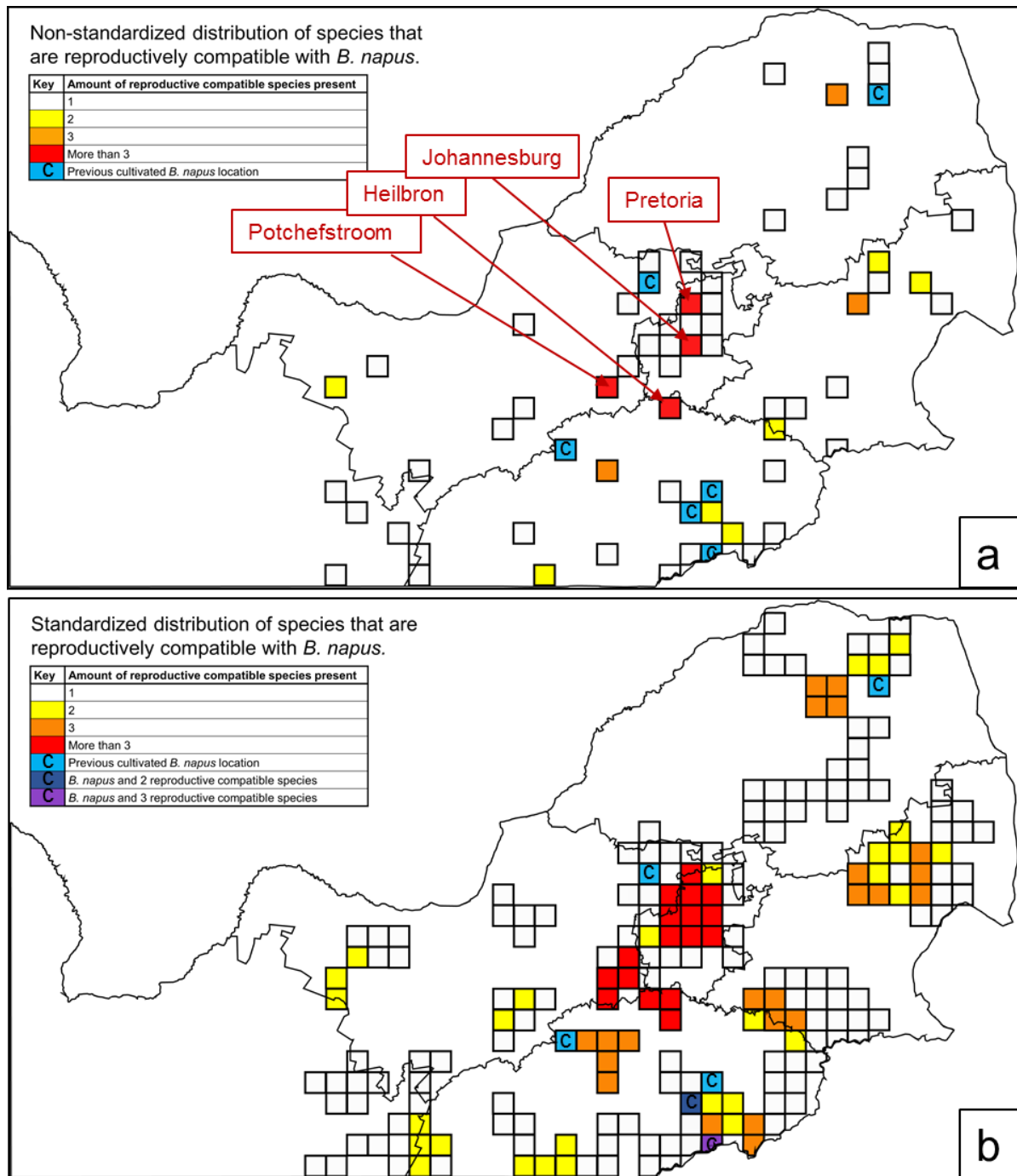


Figure 5.2: Number of reproductively compatible relatives per QDGC within the study area using non-standardized (a) and Centroid Grid profile standardized (b) data.

When looking at the QDGC's that contain the most reproductively compatible relatives, Gauteng presented the highest frequency in both the non-standardized (Figure 5.2a) and standardized (Figure 5.2b) distributions. The cities of Pretoria, Johannesburg, Potchefstroom and Heilbron are present in each of the QDGC's that contain the most reproductively compatible relatives (Figure 5.2a). The QDGC's that further contain the most reproductively compatible species are

largely present along the border of the study area, from the north-east to the south (Figure 5.2a,b). A preliminary spatial risk assessment regarding the distribution of the confirmed reproductively compatible species identified this area as the area with the highest potential for gene flow to occur between *B. napus* and its reproductively compatible relatives (Figure 5.3). This area does not coincide with bioregions, rainfall, anthropological distribution networks or any of the species, genus or tribe clusters that were determined by dendrograms (see Chapter 4).

The distribution of reproductively compatible relatives in the standardized distribution is similar to the non-standardized distribution. The standardized distribution (Figure 5.2b) of the reproductively compatible relatives is however slightly larger than the non-standardized distribution (Figure 5.2a) due to the increase in overall QDGC's as determined by the Centroid Grid profile.

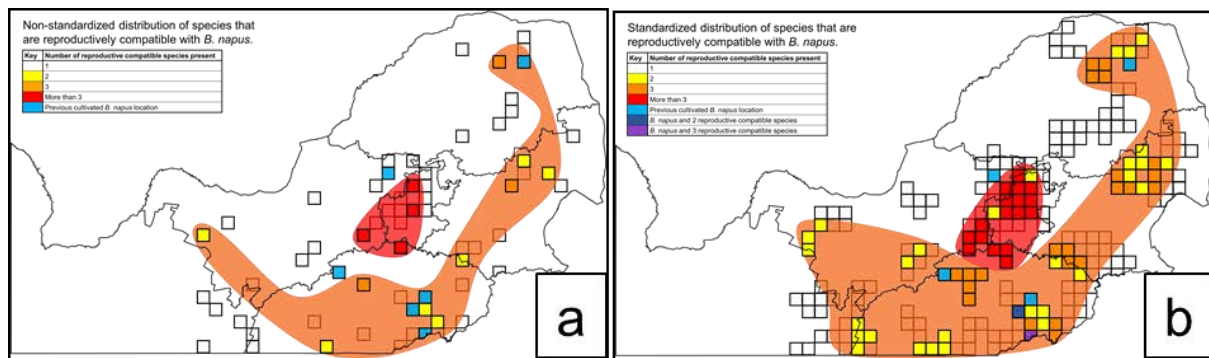


Figure 5.3: An indication of potentially high risk areas for gene flow, based on the number of reproductively compatible species present in the non-standardized (a) and standardized (b) distribution data of the Brassicaceae.

5.3.5.2 Spatial risk assessment by species rank scores

Each of the Brassicaceae species present in the study area was identified as indigenous or alien, the abundance indicated as number of specimens, the distribution (number of QDGC's) determined (Appendix B), and reproductive compatibility indicated (Chapter 4: Table 4.13). All known reproductively compatible relatives present in the study area were alien and naturalized (Appendix B). Species that were confirmed to be reproductively compatible with *B. napus* and present in the study area were assigned ranks based on prevalence, spatial overlap, gene flow rate and the presence of anthropological distribution networks (5.3.1–5.3.4) (Table 5.5).

Table 5.5: Ranking of known reproductively compatible *Brassica napus* relatives, indicating likelihood of gene flow between species in the northern regions of South Africa.

Rank	Reproductively compatible species	Prevalence	Spatial overlap	Gene flow rate	Methods of distribution	Σ	Risk score
1	<i>Raphanus raphanistrum</i>	1	1	5	1	8	10
2	<i>Brassica rapa</i>	3	4	1	2	10	9
3	<i>Diplotaxis muralis</i>	2	3	3	3	11	8
4	<i>Rapistrum rugosum</i>	4	1	9	3	17	7
5	<i>Brassica juncea</i>	6	5	2	6	19	6
6	<i>Sinapis arvensis</i>	5	6	6	5	22	5
7	<i>Brassica nigra</i>	9	9	4	8	30	4
8	<i>Eruca sativa</i>	7	8	10	7	22	3
8	<i>Raphanus sativus</i>	8	7	7	10	22	3
10	<i>Sinapis alba</i>	9	9	8	8	34	1

After the different factors were calculated to determine the rank order of reproductively compatible species, *Raphanus raphanistrum* was ranked as the species with the highest potential for gene flow to occur, followed by *B. rapa* and *D. muralis* (Table 5.5). *Raphanus raphanistrum* dominated geographically when comparing its distribution to the presence of main roads and railway lines with the 2nd ranked species, *B. rapa*, having 20 QDGC's less (5.3.2, Appendix C).

Each QDGC in the study area was inspected for the presence of reproductively compatible relatives (Appendix C) and the overall score of every QDGC was calculated based on the assigned ranks (Table 5.5) of each reproductively compatible relative present. Considering the data obtained, the non-standardized QDGC's were split into six clusters of four numbers each, and the standardized QDGC's into six clusters of six numbers each. The highest scores indicate QDGC's where the potential for gene flow to occur is the highest.

Table 5.6: Potential risk categories assigned to non-standardized QDGC's based on the cumulative species risk scores of reproductively compatible relatives.

Cumulative species risk score	Potential risk of QDGC
≥ 31	Very high
25 – 30	High
19 – 24	Relatively high
13 – 18	Medium
7 – 12	Relatively low
1 – 6	Low

Categories of potential risk for QDGC's indicate illustrate potential risk areas on non-standardized (Figure 5.4a) and standardized (Figure 5.4b) distribution maps of the study area. The high potential gene flow areas indicated in the non-standardized distribution map (Figure 5.4a) is mostly present in the same area that was initially indicated in Figure 5.3a. The standardized (Figure 5.4b) distribution of ranked species as determined per individual QDGC indicate similar areas of "high" potential gene flow throughout Gauteng, through to where the "very high" potential gene flow areas are present in the non-standardized distribution map (Figure 5.4a).

The same high potential gene flow areas in the non-standardized distribution map (Figure 5.4a) were downgraded to relatively medium and high potential gene flow areas in the standardized distribution map (Figure 5.4b). Relatively low to no reproductively compatible relatives occurred in the Northern Cape as expected (determined by the Spatial Assessment in Chapter 4).

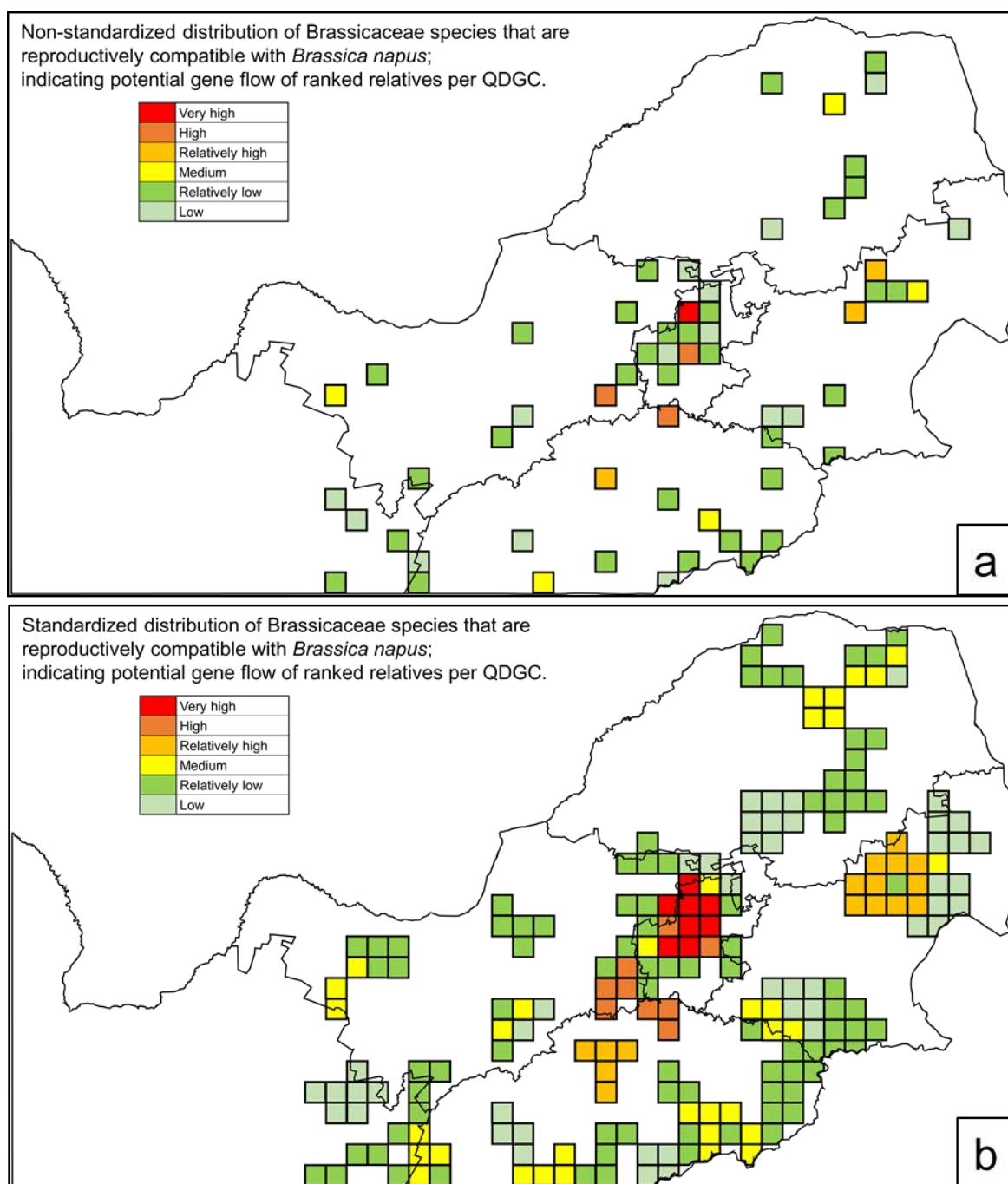


Figure 5.4: Non-standardized (a) and Centroid Grid profile standardized (b) reproductively compatible species potential gene flow risk per QDGC, as calculated from species risk scores generated from the four risk assessment method (Table 5.1).

When looking at the QDGC's that present the highest potential likelihood for gene flow to occur from the cultivation of genetically modified *B. napus*, Gauteng presented the highest gene flow risk in both the non-standardized and standardized distributions of the unranked (Figure 5.2) and ranked (Figure 5.4) reproductively compatible relatives. The cities of Pretoria, Johannesburg, Potchefstroom and Heilbron are present in each of the "very high" potential gene flow QDGC's (Figure 5.2a). The QDGC's that surround these cities and towns of "very high"

potential gene flow have been classified as “medium” or lower potential gene flow areas (Figure 5.4).

The area that was identified in this study to be the area with highest likelihood (high to very high) for gene flow to occur comprises most of Gauteng extending to parts of eastern North-West and the most northern part of the Free State (Figure 5.5). The medium to relatively high potential gene flow risk area is large and overlaps with the area where the most reproductively compatible species occur (Figures 5.2). This area extends throughout most of the eastern Free State, central Mpumalanga, southwestern North-West and eastern Limpopo (Figure 5.5).

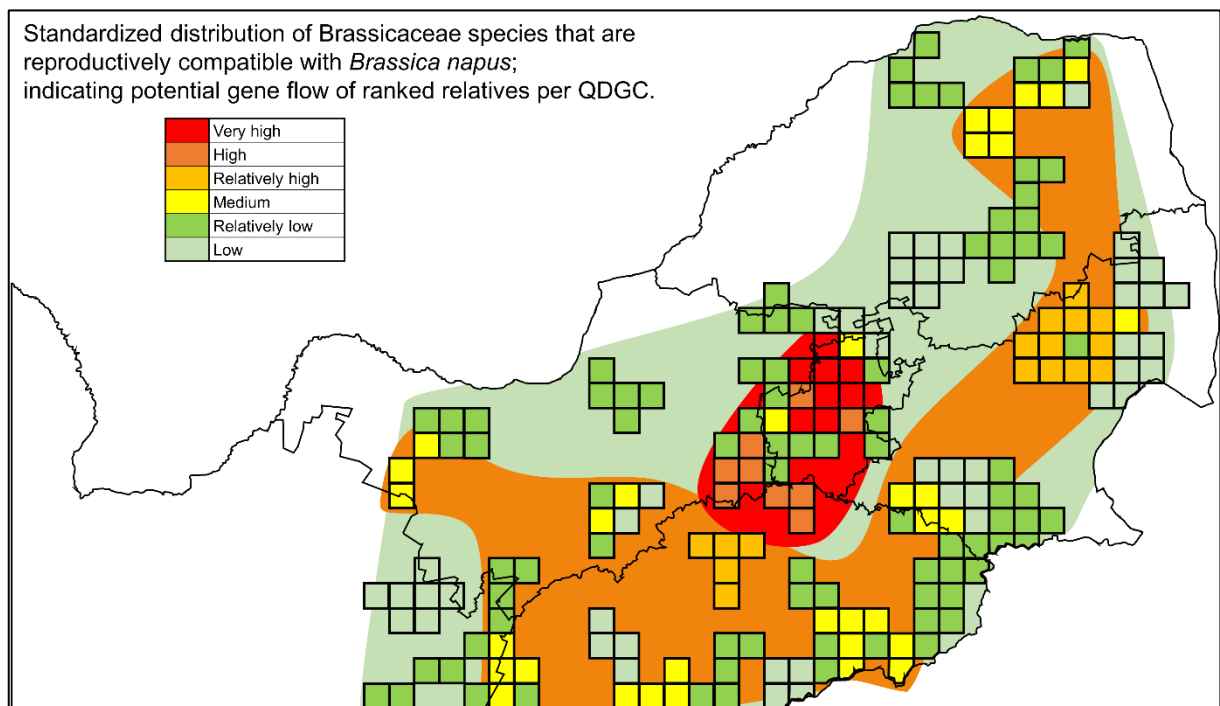


Figure 5.5: Map of the northern regions of South Africa indicating areas with different likelihoods for gene flow to occur between *B. napus* and its wild and weedy relatives.

Areas with medium to high likelihood of gene flow are present throughout the Dry Highveld Grassland and Mesic Highveld Grassland bioregions, and are also abundantly present in the Mopane and Lowveld bioregions (Figure 5.6a). Areas with a low likelihood of gene flow are situated across the Eastern Kalahari Bushveld and Central Bushveld bioregions. Regions with medium to very high likelihood of gene flow was distributed in rainfall regions with >300 mm per season, and only parts of northern Limpopo and North-West Province where the seasonal rainfall is between 100 and 300 mm (Figure 5.6b). *Bioregions and rainfall as in Chapters 3 and 4.*

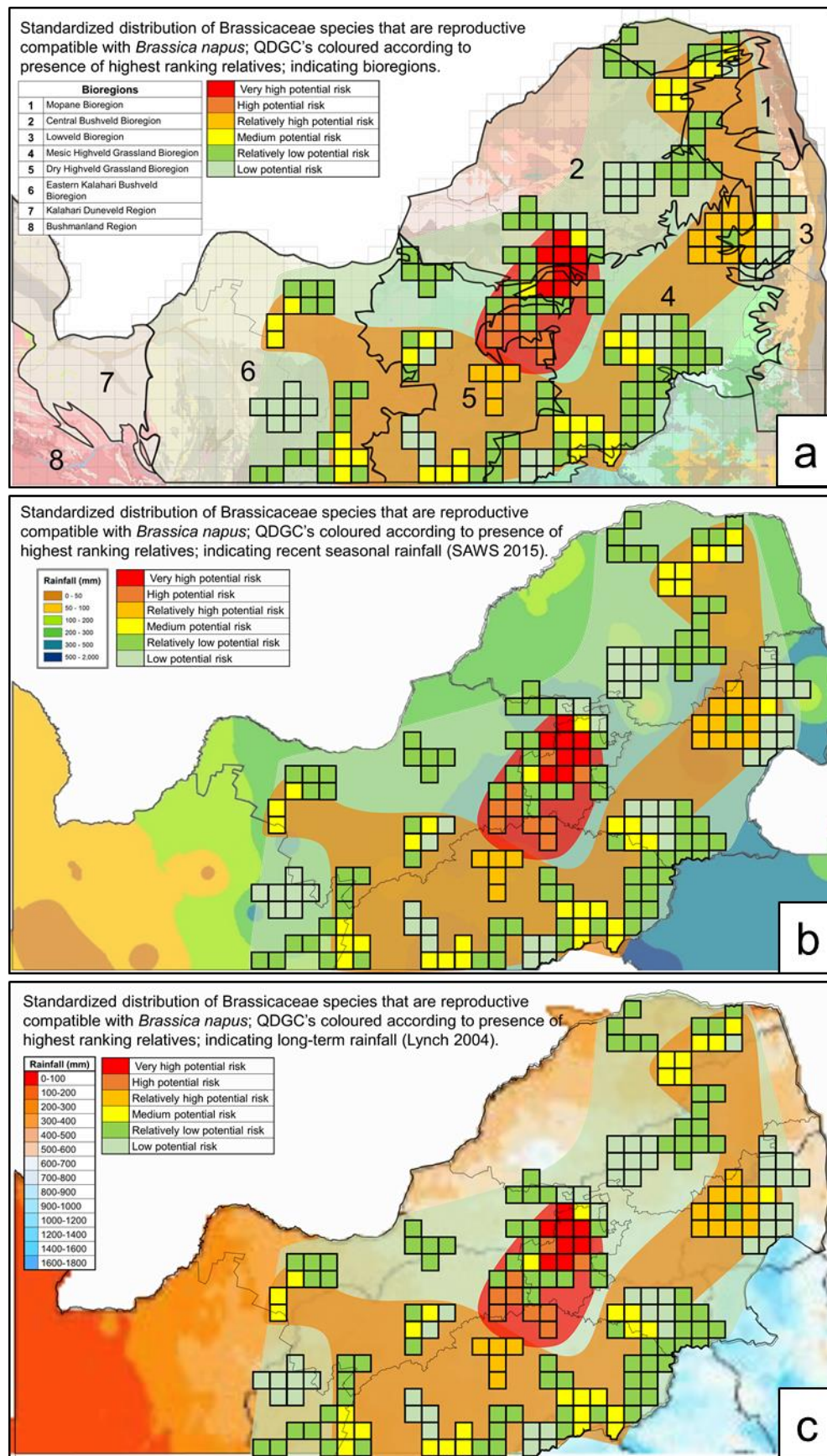


Figure 5.6: Likelihood for gene flow occur compared to bioregions (a), seasonal rainfall (SAWS 2015) (b) and rainfall between 1950 and 1999 (Lynch 2004) (c).

Between 1999 and 2000 there was an application to produce genetically modified *B. napus* in South Africa (Thomson 2002). However, this application was not approved. During 2009, Monsanto unsuccessfully applied for a field trial release of GM *B. napus* in South Africa (Stafford 2009). The reasons for non-approval were that the DNA of GM *B. napus* was considerably altered and that it provided an unknown risk (Stafford 2009). Since Monsanto withdrew their application during 2010 (ACB 2010) no further applications to introduce GM *B. napus* to South Africa has been made.

South Africa does not cultivate transgenic *B. napus* (Gruère & Sengupta 2008). Imports of genetically modified *B. napus* as food and feed were approved for South Africa during 2001 (Gruère & Sengupta 2008; ISAAA 2014). The imported *B. napus* seed encodes for glufosinate herbicide tolerance, male sterility, fertility restoration and antibiotic resistance (Chapter 2, Table 1.2) (ISAAA 2014) (see Chapter 6 for further information on specific traits).

5.3.6 Risks and consequences

Introgression, the permanent fixation of a gene or transgene that encodes for herbicide tolerance, for example, into a population after gene flow and hybridization may increase the weediness of a specific plant population by improving its competitiveness under certain conditions (Gueritaine *et al.* 2002). Introgression is not always morphologically evident but may be present molecularly (Petit 2004). It is important to consider that hybridization may not always lead to introgression, and that low hybridization rates and reduced hybrid fitness may simply slow down the process of introgression (Hails & Morley 2005). The fitness of the F₁ generation determines the success involved with introgression of a transgene into a wild and weedy relative population (Warwick *et al.* 2008).

Seed of GM crops could potentially escape into the environment where they can hybridize with wild relatives. *Brassica napus* hybridizes freely with *B. juncea* and *B. rapa* in Japan (Bauer-Panskus *et al.* 2013). *Brassica napus* is frequently distributed by anthropological transportation networks and in Canada escaped seeds have been reported to form populations that hybridize with *B. rapa* (Bauer-Panskus *et al.* 2013). Aono *et al.* (2006) found *B. napus* seeds that had escaped into the environment but whilst they could not determine whether introgression had occurred to its wild and weedy relatives at that time, they observed that different varieties of *B. napus* had hybridized. Hybridization between different populations may allow for transgene stacking such as that reported in Canada where the F₁ generation encoded for multiple-trait resistance to herbicides (Beckie *et al.* 2003; Hall *et al.* 2000; Simard *et al.* 2002). Hybridization between *B. napus* and *B. rapa* has indicated that transgenes may introgress into an adjacent relative population irrespective of herbicide use (Kwit *et al.* 2011; Warwick *et al.* 2008).

Since volunteer populations may contain traits from both the wild and weedy populations as well as the crop, volunteers may have beneficial fitness traits such as longevity which is characteristic of both *B. napus* and *B. rapa* (Kwit *et al.* 2011; Légerè 2005; Reagon & Snow 2006; Simard *et al.* 2002). Alternatively, speciation may occur when genes from the crop species and its wild and weedy relatives combine to create traits that are not present in either of the parent populations (Hails & Morley 2005).

It is assumed that the transgene which is maintained in each population is passed on to the next generation (Quist & Chapela 2001). A study conducted in Canada, France and Japan reported that the incidence of volunteer *B. napus* plants decreased over time but that volunteer plants were still present after five years (Simard *et al.* 2002), six years (Bauer-Panskus *et al.* 2013; Warwick *et al.* 2008) and even eight years (Pessel *et al.* 2001) after initial cultivation. When seed of volunteer *B. napus* persist in the seedbank its plants may become weeds in subsequent cultivated crops. This was reported in crops such as wheat, (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), rye (*Secale cereal* L.), maize (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr) (Simard *et al.* 2002). Quist & Chapela (2001) further indicated that gene flow between transgenic and non-transgenic maize may lead to the transgene being present in previously non-transgenic maize plants. If transgenic *B. napus* persists and volunteer populations are produced any possible cultivated non-transgenic *B. napus* may be contaminated similarly. Furthermore, transgenic volunteer *B. napus* may become weeds in the following seasons if it acquired a trait that gives it a competitive advantage over plants occurring within the same locality.

The ability of crop species to form volunteer populations may be a reason for concern. Reagon & Snow (2006) identified sunflower (*Helianthus annuus*) volunteer populations as a major escape route for transgenes from sunflower crop plants. Hall *et al.* (2000) reported similar observations regarding *B. napus* and its volunteers. Due to the cultivated and volunteer population being the same species, their phenologies overlap considerably (Reagon & Snow 2006). These volunteer populations may act as genetic bridges to further populations (Warwick *et al.* 2008).

Warwick *et al.* (2003) assessed the actual gene flow rates between *B. napus* and four of its relatives. *B. rapa*, *Erucastrum gallicum*, *Sinapis arvensis* and *Raphanus raphanistrum*, in greenhouse and field experiments. The study found that gene flow between *B. napus* and *E. gallicum*, *S. arvensis* and *R. raphanistrum* were unlikely, but that gene flow from *B. rapa* is likely to occur (Warwick *et al.* 2003). Gene flow between *B. napus* and *B. rapa*, and *B. rapa* volunteer populations was reported by Warwick *et al.* (2003). In this study *B. rapa* was ranked as the reproductively compatible species with the highest gene flow rate but was ranked second

overall (Table 5.1) due to the high values of *R. raphanistrum* at each of the three other categories (prevalence, spatial overlap and anthropological distribution) which resulted in it being assigned a rank of 1.

5.4 Summary

Gene flow from a transgenic crop to its wild and weedy reproductively compatible relatives could have several negative consequences. The most recognized of these negative consequences is the loss of genetic diversity (Vilà *et al.* 2000) and the threat of development of weeds with increased competitive abilities.

The aim of the spatial risk assessment was to determine which Brassicaceae species present the highest potential and likelihood for gene flow occur, and identify spatial areas where the risk for gene flow to occur between *B. napus* and its reproductively compatible relatives is highest. Seventy-six Brassicaceae species were identified within the northern provinces of South Africa of which ten were confirmed reproductively compatible with *Brassica napus*.

Differences in phenologies, pollination and seed dispersal patterns (Vilà *et al.* 2000) may present further barriers to gene flow, but has been overcome by *B. napus* and its wild and weedy relative populations. An increase in anthropological distribution of reproductively compatible relatives is an important factor that could influence the likelihood of gene flow to occur (Vilà *et al.* 2000) through seed dispersal. Anthropological activity allows natural barriers to be overcome through disturbance and fragmentation of habitats, thus increasing the success and distribution of hybrid species (Vilà *et al.* 2000).

These factors were taken into account to identify and rank the species that are reproductively compatible and most likely to exchange genetic material with *B. napus*. We have identified *Raphanus raphanistrum* (based on available data) as the species that presents the highest likelihood for gene flow to occur between it and *B. napus*, followed by *B. rapa* and *Diplotaxis muralis* based on prevalence, spatial overlap, gene flow rate and means of distribution. We have further identified the area that present the highest potential gene flow risk to be along the eastern to south-eastern border of the study area, with an increase in the likelihood for gene flow to occur where there are anthropological activities and a higher amounts of rainfall.

To assess the risk after gene flow occurred from transgenic *B. napus* to its volunteers and wild and weedy relatives, extensive knowledge on hybrid ecology and biology is required (Vilà *et al.* 2000). A risk assessment should therefore be conducted considering realistic agricultural and

ecological conditions (Vilà *et al.* 2000; Warwick *et al.* 2008). This study provides information on the likelihood of gene flow to occur between GM *B. napus* and its wild and weedy relatives and therefore contributes to future risk assessments regarding GM *Brassica* species in South Africa. Consequences of gene flow from GM *B. napus* are stated in Chapter 6, along with possible mitigation strategies.

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Chapter 6: Conclusion and Recommendations

6.1 Phytogeography of the Brassicaceae (Chapter 4)

Baseline data for the study was specimen accessions collected from various major herbaria (4.3.1.1). The problem posed by using specimen data is a collector's bias (Appendix C; Figure 4.7) that was first effectively resolved by standardizing the species to absence/presence data. To simplify the distribution data across the study area, QDGC's (Quarter Degree Grid Cells) were used to divide the study area into smaller areas. Data was split into six different data sets: 1) non-standardized species, 2) Centroid Grid profile standardized species, 3) non-standardized genera, 4) Centroid Grid profile standardized genera, 5) non-standardized tribes and 6) Centroid Grid profile standardized tribes (Table 4.1). Standardizing the absence/presence species data via the Centroid Grid profile increased the number of QDGC's (that contain species) by distributing species across the study area based on similarity of vegetation units (Mucina & Rutherford 2006) (4.3.1.2) (Figures 4.4, 4.5). This final standardization increased the species present in potentially undersampled (due to large surface area and/or low population densities) regions (Figure 4.7).

Phytochoria for each of the six different data sets were obtained with PRIMER and Bray-Curtis similarity dendrograms. SIMPROF was applied to each dendrogram, followed by adding a resemblance slice to distinguish different clusters with similar species composition (Figures 4.9, 4.13, 4.19, 4.24, 4.30, 4.33). These clusters were inserted into PRIMER to obtain NMDS graphs and the dominant species, genus and tribes were respectively identified for each cluster in each data set (Figures 4.10, 4.14, 4.20, 4.23, 4.29, 4.34). All species that occurred in more than 75% of the QDGC's in each cluster was identified as dominant. *Heliophila rigidiuscula*, *Lepidium africanum* and *Nasturtium officinale* were the most frequently dominant in the three non-standardized data sets, and *Erucastrum austroafricanum*, *Lepidium africanum*, *Rorippa fluviatilis* var. *fluviatilis* and *Sisymbrium burchellii* var. *burchellii* in the three standardized data sets (Tables 4.4–4.9).

The standardized data sets presented an improved distribution for the Brassicaceae. As such, these figures (species, genera and tribes) were superimposed on bioregions (Mucina & Rutherford 2006) (Figures 4.16, 4.27, 4.36), seasonal rainfall for a single year (SAWS 2015) and rainfall over the course of 50 years (between 1950 and 1999) (Lynch 2004) (Figures 4.17, 4.26, 4.37) to indicate factors that may possibly influence the distribution of the Brassicaceae. Bioregions and rainfall were mostly correlated so that a specific bioregion covers an area where a specific amount of rainfall is present. Brassicaceae occur throughout the study area with the

most species present in high rainfall areas (e.g. Mesic Highveld Grassland bioregion), and little to no species present in low rainfall areas (e.g. Bushmanland region).

ANOSIM and SIMPER analyses were conducted to determine the similarity between the clusters within each of the data sets and as comparison between the different data sets (Tables 4.10–4.12). The non-standardized species distribution was the most dissimilar and the Centroid Grid profile standardized tribe distribution the least, albeit still dissimilar (68.14%) (Table 4.11).

Ten Brassicaceae species were identified to be reproductively compatible with *B. napus* (*B. juncea*, *B. nigra*, *B. rapa*, *Diplotaxis muralis*, *Eruca sativa*, *Raphanus raphanistrum*, *R. sativus*, *Rapistrum rugosum*, *Sinapis alba* and *S. arvensis*) (Table 4.13). *B. rapa*, *D. muralis*, *Raphanus raphanistrum*, *Rapistrum rugosum* and *S. arvensis* were dominant in the species cluster distributions (Tables 4.4, 4.5), and *Brassica*, *Diplotaxis*, *Raphanus*, *Rapistrum* and *Sinapis* in the genera cluster distributions (Tables 4.6, 4.7). All ten the reproductively compatible species are within tribe Brassicaceae, which was dominant in clusters within both tribe distributions (Tables 4.8, 4.9). The reproductively compatible relatives occur abundantly where rainfall is highest (Figure 4.40) and in associated bioregions (Figure 4.39).

Five phytochoria were determined by isolating the standardized species clusters that contain reproductively compatible relatives. These are 1) low rainfall, 2) high rainfall, 3) Soutpansberg, 4) Eastern Waterberg, and 5) Drakensberg foothills (Figures 4.41, 4.42).

6.2 Spatial risk assessment of canola (Chapter 5)

Each of the ten species that are reproductively compatible with *B. napus* was weighed against four factors as a means to determine the overall risk that may be associated with each. Prevalence, an indication of overall distribution throughout the study area in both non-standardized and standardized distributions, identified *Raphanus raphanistrum* as the reproductively compatible species that is most prevalent, followed by *Diplotaxis muralis* and *B. rapa* (Table 5.1).

The QDGC's where *B. napus* had previously been cultivated were identified in the phytogeography of the Brassicaceae (Chapter 4) so that the spatial overlap between it and its reproductively compatible relatives could be determined. *Raphanus raphanistrum* and *Rapistrum rugosum* was identified as the species that overlapped the most with *B. napus*, followed by *D. muralis* (Table 5.2).

The gene flow rate of each species was obtained from literature, as it was not determined within the study. Number of successes and failures to hybridize were obtained from FitzJohn *et al.*

(2007) and were used to calculate the proportion of successes to failures as an indication of gene flow rate. *B. rapa* was identified as the species with the highest gene flow rate, followed by *B. juncea* and *D. muralis* (Table 5.3).

The distribution of Brassicaceae seed occurs frequently due to anthropological activity (Barnaud *et al.* 2013; Crawley & Brown 1995; Reagon & Snow 2006). As such, the presence of railways and main roads in the same and surrounding reproductively compatible relative QDGC's were identified as potential methods for the further distribution of these species (Figure 5.1). *Raphanus raphanistrum* was identified as the species that had the highest potential to distribute because of anthropological activities, followed by *B. rapa* and *D. muralis* (Table 5.4).

The number of reproductively compatible relatives were plotted to first determine areas that may be of risk (Figure 5.2). Gauteng, to Potchefstroom in the North-West and Heilbron in the Free State (Figure 5.2) was identified as having the highest potential to present the highest risk for gene flow in the study area, followed by a large area that stretches from eastern Limpopo along the eastern edge of the study area, through the entire Free State and covering half of the North-West province (Figure 5.3). Risk scores identified *R. raphanistrum* as the species that presents the highest overall risk for gene flow to occur to *B. napus*, followed by *B. rapa* and *D. muralis* (Table 5.5).

Raphanus raphanistrum, the species that presents the highest potential risk for gene flow to occur between cultivated canola species in the northern provinces of South Africa, is one of the worst agricultural weeds in the world (Barnaud *et al.* 2013). It is known to invade disturbed habitats, roadsides and compete with agricultural crops (Barnaud *et al.* 2013; Snow & Lesley 2005) (Figure 6.1). As with *B. napus*, its seeds may germinate after long periods of time in the seedbank (Barnaud *et al.* 2013; Snow & Lesley 2005). *R. raphanistrum*, otherwise known as wild radish, has an annual rapid life cycle, is allogamous, diploid, pollinated by insects and self-incompatible (Barnaud *et al.* 2013; Snow & Lesley 2005; Holm *et al.* 1997, Warwick & Francis 2005). It occurs throughout most of South Africa, with the exception of areas where there is low rainfall (McGeoch *et al.* 2009).

The risk scores obtained per species were utilized to calculate the total risk score for every QDGC in the study area by adding the individual risk scores of every reproductively compatible relative in the QDGC. These total scores were added to a scale (Table 5.6) and plotted on a map to identify the relatively same regions (Figure 5.5) as potentially determined using only abundance data (Figure 5.3). The area presenting the highest risk is distributed throughout most of Gauteng and slightly south of it, the relatively high risk area is distributed along the eastern and south end of the study area, starting at Limpopo and through the entire Free State

and the southern half of the North-West province, and the low risk, but still a risk area, covers almost the entire study area, with the exception of the Northern Cape (Figure 5.5).

All of the locations where *B. napus* has previously been cultivated is included within the relatively high risk area. The risk areas were further superimposed on bioregions and rainfall to illustrate that both the high risk areas are present in the northern Lowveld, Mesic Highveld Grassland, Dry Highveld Grassland and east Eastern Kalahari Bushveld bioregions, which covers an area where seasonal and long-term annual rainfall was higher than 200 mm (Figure 5.6). The areas of high risk indicate that the Brassicaceae require more than 200 mm rainfall to thrive, which is a fact further confirmed by farmers that need plenty of irrigation to cultivate *B. napus* (DAFF 2010). The irrigation of this crop species will present a further niche for reproductively compatible relatives to occur within, and creates thus further potential risk.

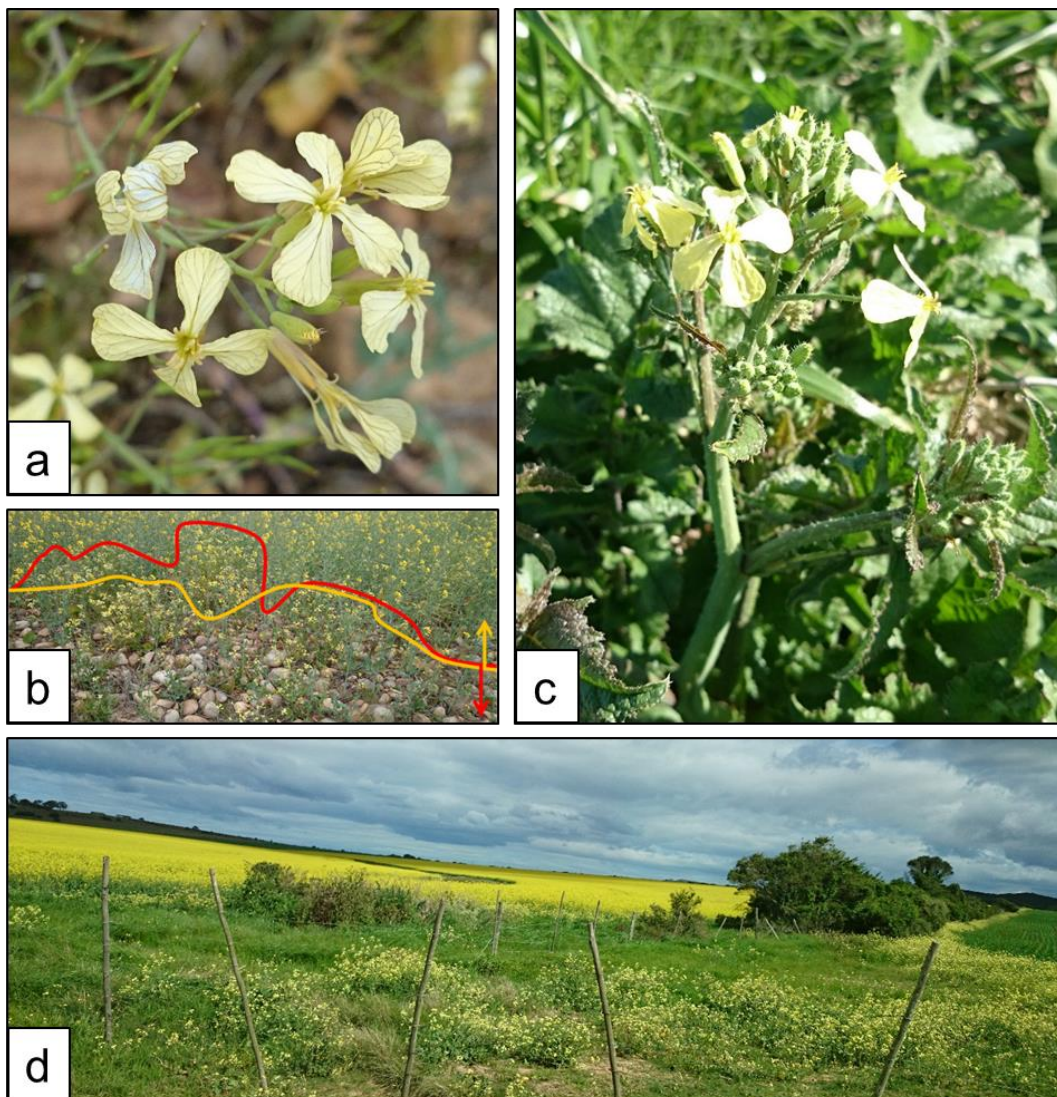


Figure 6.1: *Raphanus raphanistrum* flower (a), presence in disturbed environments (b), weedy appearance (c) and presence along *Brassica napus* agricultural fields (d). Species above the yellow line is *B. napus* and beneath red line is *R. raphanistrum*. (Photos by B. Greyvenstein.)

6.3 Recommendations for transgenic canola cultivation

When reproductively compatible species occur in the same geographical area, gene flow will occur (Warwick *et al.* 2003). Gene flow from a transgenic crop species may introduce transgenes into its wild and weedy relatives. The probability that a specific advantageous trait will develop naturally within any given population is much lower than in cases where it is acquired through gene flow and hybridization (Warwick *et al.* 2003). Transgenes encode for transgenic traits that may be beneficial to plants by allowing stresses and barriers to be overcome. Transgenic traits may however persist in future generations irrespective of whether the transgene increases the fitness of the crop (Pessel *et al.* 2001; Warwick *et al.* 2008). To determine the actual consequences of gene flow the specific transgenic traits should be considered. Certain traits may increase the potential for the F₁ generation to increase in weediness and invasiveness (Warwick *et al.* 2008). Ellstrand & Schierenbeck (2000) described an event where gene flow occurred between cultivated biennial beet root and its annual wild beet relative. The annual F₁ generation was morphologically similar to the crop but formed woody roots that cannot be used for commercialization (Ellstrand & Schierenbeck 2000). Annual *B. napus* species were cultivated in Japan, but after hybridizing with its wild and weedy *B. rapa* relatives the F₁ generation contained perennial hybrids (Bauer-Panskus *et al.* 2013). Hybrid resistance to biotic environmental disturbances such as pathogens or herbivores may further increase the fitness of these hybrid species (Vilà *et al.* 2000).

Hybrid species encoding for transgenic traits may have a competitive advantage (Warwick *et al.* 2003) when the specific transgenic trait is introgressed into the population and it outcompetes non-transgenic natural-occurring plants in the same location. The increased distribution of these competitive weeds may reduce the growth of and replace populations that occur within the same vicinity (Vilà *et al.* 2000). The development of weeds with increased competitive abilities can however only be realised when a specific environmental stress is present that benefits the newly hybridized weed species above the populations present within the same locality. For example, a weedy *Brassica* species that has acquired a herbicide tolerant trait will most likely only have a competitive advantage over its herbicide susceptible counterparts within the same locality if herbicide is applied to the population. For this reason it may be said that transgenic traits may or may not enhance a hybrid species' fitness.

Since gene flow between GM and wild relatives could have unintended effects, it is important to develop strategies to prevent or at least limit the likelihood of gene flow. The development of strategies or barriers between reproductively compatible relatives will prevent hybridization between different populations, or even within different individuals in the same population. Methods of mitigation that are in place to attempt the prevention of hybridization include the

presence of guard or border rows, male sterility, alteration of flowering times, and post-zygotic barriers (Beckie *et al.* 2003; Kwit *et al.* 2011). Prevention of transgene flow by using the above mentioned strategies on their own will not be effective to prevent transgene escape, despite the fact that the chance of such an event to occur is one in a thousand or one in a million (Kwit *et al.* 2011). It is therefore recommended that multiple mitigation strategies are combined to minimize and actually prevent transgene escape from a transgenic crop to its wild and weedy relatives. There are also various strategies that could be employed to manage transgenes once they escape and become introgressed into wild or weedy populations. Such strategies include rotation of herbicides with different modes of action and crop rotation.

Different herbicide types. The genetically modified *B. napus* varieties that are imported into South Africa for food and feed contain transgenes encoding for glufosinate herbicide tolerance (ISAAA 2014). Glufosinate herbicide tolerance can be managed by using weed-control practices or herbicides that these transgenic varieties of *B. napus* do not encode for (Warwick *et al.* 2003). The presence of weedy *B. napus* volunteers and relatives may further be reduced by switching the type of in-crop herbicide used to herbicide containing chemicals that the weeds are not resistant to (Gulden *et al.* 2003; Nabors 2004).

Crop rotation. The presence of weedy transgenic species within a field may be reduced by using crop rotation (Nabors 2004). *Brassica napus* is used as a rotation crop in the Western Cape Province of South Africa for protection of cereal crops such as wheat (*Triticum aestivum* L.) against soil diseases and weeds (Hardy 2004; Tewoldemedhin *et al.* 2006). The value and use of *B. napus* as rotation crop could be affected if its weedy relatives become resistant to a particular herbicide which will imply that it cannot be chemically controlled in follow-up non-*Brassica* crops. Rotations with different crops encoding for the same transgenic herbicide resistance trait may allow multiple volunteers from different crops to be resistant to the herbicide (Légère 2005). Gulden *et al.* (2003) suggested that crop species in which herbicides may be used that can control volunteer *B. napus* be planted directly after a *B. napus* crop.

Border areas. In Canada, a gene flow assessment was conducted between neighbouring commercial fields during 1999 and 2000 (Beckie *et al.* 2003). The study suggested that the presence of guard or border rows between fields would not prevent gene flow. Even though the levels of gene flow decreased considerably up to 50 m away from the field, little change occurred between 50 and 400 m (Beckie *et al.* 2003). This same phenomenon had previously been identified by Quist & Chapela (2001), Rieger *et al.* (2002) and Staniland *et al.* (2000): gene flow and introgression occurs at a higher rate when the populations are in close proximity and decreases the further away from the source (in this case cultivated *B. napus*) the wild and weedy population is. Rieger *et al.* (2002) named this phenomenon “edge effect”.

Male sterility. For transgene flow to occur between a crop species and its wild and weedy relatives, the pollen of one of the species should contain the transgene (Kwit *et al.* 2011). By preventing pollen formation, the most direct method of distribution of these transgenes to relative populations is eliminated (Kwit *et al.* 2011). Decreased male fertility is achieved by genetic male sterility or cytoplasmic male sterility (Kwit *et al.* 2011). A recent study indicated that the use of sub-lethal doses of glyphosate herbicides on *B. napus* suppresses fertility and alters flowering times, but that it may give rise to evolution of resistant weeds (Londo *et al.* 2014).

Two of the four genetically modified *B. napus* varieties that is imported to South Africa for food and feed purposes encodes for male sterility (*barnase*) and fertility restoration (*barstar*) (Chapter 2: Table 2.2) (ISAAA 2014). Both these traits are obtained from *Bacillus amyloliquefaciens* (ISAAA 2014). The former encodes for male sterility by interfering with RNA production in the tapetum cells of the anther and also through preventing functionality, while the latter (fertility restoration) suppresses the effect of the former (male sterility) on the tapetum cells of the anther (ISAAA 2014; Kempe & Gils 2011). The use of male sterility as barrier to gene flow between *B. napus* and its relatives in South Africa is not an option since this crop has to be fertilized to produce seeds needed for oil-extraction (DAFF 2013). Fertility is restored to ensure optimal yield in crops that are cultivated for their seed, such as *B. napus* (Kempe & Gils 2011).

Flowering times. Different flowering times of *B. napus* and its wild and weedy reproductively compatible relatives would prevent hybridization between these species (Kwit *et al.* 2011). Alteration on flowering periods for *B. napus* may, however, decrease the seed yield due to the flowering period falling short of the optimal time required for maximum production (Kwit *et al.* 2011). Furthermore, different individuals in the same population may flower at different times so that gene flow to an adjacent relative population may still occur, albeit in a very short time (Kwit *et al.* 2011).

Genetic barriers. The specific position of a transgene on the genotype of *B. napus* may influence the overall chance of the transgene to persist in a reproductively compatible population. Linkage disequilibrium occurs when a transgene is placed in loci that have low probability of being introgressed into wild and weedy populations due to the lower fitness of that part of the genome (Kwit *et al.* 2011). Even though the linkage disequilibrium may lower the chance for transgenes to be transferred to another population, non-transfer cannot be guaranteed since recombination of the genome may occur frequently (Kwit *et al.* 2011). Another post-zygotic barrier that can be used in future is the phenomenon of different ploidy levels between different populations (Kwit *et al.* 2011). When different species populations have different ploidy levels, transgene introgression may be suppressed when hybridization occurs

(Sandhu *et al.* 2010). The ability of *B. napus* to hybridized with its reproductively compatible relatives, especially with *B. rapa*, is however not affected by different ploidy levels (Kwit *et al.* 2011), and this strategy can therefore not be used as a mitigation strategy for this crop species.

Of the four genetically modified varieties of *B. napus* that is imported to South Africa for food and feed purposes, three contain a gene that encodes for antibiotic resistance (ISAAA 2014). This gene is identified as a selection marker or reporter and encodes for an enzyme that may be relatively easy to distinguish in a wild or weedy population (ISAAA 2014).

A risk assessment that focuses on ecological and agronomic factors is especially important if transgenic *B. napus* is to be cultivated for biofuel production (McGeoch *et al.* 2009). If *B. napus* is to be produced as biomass for biofuel production the area for cultivation of the crop will be increased (McGeoch *et al.* 2009). Understanding distribution methods and attributes of transgenic and non-transgenic *B. napus* will further our ability to understand and react to unexpected consequences relating to the large-scale cultivation of transgenic *B. napus* varieties (Pessel *et al.* 2001).

Several possible negative consequences of the adoption of transgenic *B. napus* within the northern provinces of South Africa have been identified. These included ecological and agronomic consequences of gene flow and introgression into wild and weedy relatives growing in anthropological disturbed sites and natural environments. Further investigation into the reproductive compatibility and gene flow rates of the indigenous Brassicaceae relatives in South Africa is essential in order to improve the overall ecological risk assessment for *B. napus*.

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Appendix A: Glossary

Allogamous – Species that cross-pollinate and consequently present more variability.

Anthropogenic – Caused or affected by human activity or influences.

Barnase – A barnase ribonuclease (RNase) enzyme obtained from *Bacillus amyloliquefaciens* that encodes for male sterility by preventing RNA to be produced in anther tapetum cells; see gene male sterility.

Barrier – A biotic or abiotic mechanism preventing the exchange of genetic material.

Barstar – A barnase ribonuclease (RNase) inhibitor obtained from *Bacillus amyloliquefaciens* that encodes for fertility restoration by preventing the effect of barnase on anther tapetum cells.

Border areas – An area surrounding a crop where no crop species are cultivated. In this study a border area is a means to minimize pollen movement.

Bridge species – see Genetic Bridge Species.

Canola – *Brassica napus* or *B. rapa* that contain less than 2% erucic acid and defatted seed meals containing less than 30 micromoles per gram of aliphatic glucosinolates. The focus was placed on *B. napus* in this study.

Cultivar – When specific traits are selectively chosen for the F₁ generation.

Edge effect – Species occurring at the edges of a source field have higher stress resistances due to the higher exposure to stress factors e.g., herbicides. Resistance to stress factors declines as distance increases away from the source field.

F1 hybrids – The first hybrid generation after gene flow between two separate plant populations occurred.

Fitness – The ability of a species to survive and reproduce effectively under specific conditions, and its proportionate contribution to the formation of the next generation.

Gene male sterility – see barnase for an example of a *B. napus* variety that is imported to South Africa for food and feed purposes.

Genetic Bridge Species (GBS) – A term given to volunteers, different varieties or relatives of a species that serve as a “bridge” to carry genes and transgenes from one population to another.

Herbicide tolerance – Genetic modification to cultivated plant species by introducing genes that allow a plant not to be affected by herbicides. Varieties of *Brassica napus* that are imported to South Africa for food and feed purposes contain phosphinothricin N-acetyltransferase (PAT) enzyme named “bar” which is obtained from *Streptomyces hygroscopicus* and encodes for the elimination of herbicidal activity of glufosinate (phosphinothricin) herbicides by acetylation.

Hybrid – The name given to an individual belonging to the F1 generation population as a result of hybridization between two different species.

Hybridization – The combination of genes from two separate plant populations of either different species or different varieties of the same species.

Introgression – The permanent presence of a specific trait from one population to another through hybridization and backcrossing over multiple generations.

Invasiveness – Tendency of a population to disperse to and establish at alternative locations. In this dissertation, the term implies a negative connotation.

Linkage disequilibrium – Transgenes that are integrated into alleles that occur less frequent may be lost during recombination of the genome and thus not carried over to the F1 generation.

Loci – The specific segment in a chromosome expressing variation in a specific trait.

Male sterility – see Gene male sterility, barnase.

Null hypothesis (H_0) – There is no significant differences between collection areas (in this case, QDGC's) so that each collection area is a duplicate of every other collection area.

Orographic precipitation – Rainfall that is caused when moist air is lifted by an orographic lifting mechanism, such as a mountain range.

Outbreeding depression – A lowering in fitness of the recipient population because of gene flow from a source population because of unfavourable gene recombinations.

Ploidy – The number of chromosome pairs within a single cell nucleus.

Stresses – Abiotic (physical or chemical) and biotic (biological e.g. insects and disease) factors that the plant may be exposed to.

Tapetum – The innermost layer of pollen sac wall cells.

Weed – Plant species that possess generally anthropologically unwanted traits that allow these plants to thrive in anthropologically disturbed environments such as agricultural fields or gardens.

Weediness – Traits that are associated with weed plant species. See Weed.

Weedy relative – Species classified within the same family that occur in the same, or border, vicinity and are dependent upon anthropological influence.

Wild relative – Species classified within the same family that occur in natural environments and are not dependent upon anthropological influence.

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Appendix B

Species list of Brassicaceae in the study area. S, specimens; Q, quarter degree grid cells.

Tribe	Genus	Species (* = alien species)	n S	n Q
Alysseae DC.	<i>Alyssum</i> L.	* <i>A. minutum</i> Schltld. ex DC.	1	1
	<i>Lobularia</i> Desv.	* <i>L. maritima</i> (L.) Desv.	4	2
Anchonieae DC.	<i>Matthiola</i> R.Br.	* <i>M. incana</i> (L.) W.T.Aiton	1	1
		<i>M. species</i>	5	5
		<i>M. torulosa</i> (Thunb.) DC.	6	4
Brassicaceae DC.	<i>Brassica</i> L.	* <i>B. elongata</i> Ehrh. subsp. <i>elongata</i>	5	2
		* <i>B. juncea</i> (L.) Czern.	5	5
		* <i>B. nigra</i> (L.) W.D.J.Koch	2	2
		* <i>B. rapa</i> L.	17	12
		* <i>B. species</i>	5	5
	<i>Diplotaxis</i> DC.	* <i>D. muralis</i> (L.) DC.	29	14
	<i>Eruca</i> Mill.	* <i>E. sativa</i> Mill.	6	3
	<i>Erucastrum</i> (DC.) C.Presl	<i>E. austroafricanum</i> Al-Shehbaz & S.I.Warwick	71	39
		<i>E. griquense</i> (N.E. Br.) O.E. Schulz	26	22
		<i>E. species</i>	1	1
		<i>E. strigosum</i> (Thunb.) O.E. Schulz	26	21
	<i>Raphanus</i> L.	* <i>R. raphanistrum</i> L.	41	16
		* <i>R. sativus</i> L.	3	2
	<i>Rapistrum</i> Crantz	* <i>R. rugosum</i> (L.) All.	15	12
	<i>Sinapis</i> L.	* <i>S. alba</i> L.	2	2
		* <i>S. arvensis</i> L.	7	7
Camelineae DC.	<i>Camelina</i> Crantz	* <i>C. rumelica</i> Velen.	2	1
	<i>Capsella</i> Medik.	* <i>C. bursa-pastoris</i> (L.) Medik.	35	17
	<i>Turritis</i> L.	* <i>T. glabra</i> L.	5	3
Cardamineae Dumort.	<i>Armoracia</i> P. Gaertn., B. Mey. & Scherb.	* <i>A. rusticana</i> P. Gaertn., B. Mey. & Scherb.	1	1
	<i>Cardamine</i> L.	<i>C. africana</i> L.	40	15
		* <i>C. flexuosa</i> With.	1	1
		* <i>C. hirsuta</i> L.	3	3
		* <i>C. impatiens</i> L.	3	-
	<i>Nasturtium</i> R.Br.	* <i>N. officinale</i> R.Br.	71	28
	<i>Rorippa</i> Scop.	<i>R. fluviatilis</i> (E.Mey. ex Sond.) Thell. var. <i>caledonica</i> (Sond.) Marais	25	15
		<i>R. fluviatilis</i> (E.Mey. ex Sond.) Thell. var. <i>fluviatilis</i>	41	26
		<i>R. madagascariense</i> (DC.) H. Hara	2	2
		<i>R. nudiuscula</i> (E. Mey. ex Sond.) Thell.	49	28
Descurainieae Al-Shehbaz, Beilstein & E.A. Kellogg	<i>Descurainia</i> Webb&Berthel.	* <i>D. sophia</i> (L.) Webb ex Prantl	1	1
Erysimeae Dumort.	<i>Erysimum</i> L.	* <i>E. cheiri</i> (L.) Crantz	1	1
Heliophileae DC.	<i>Heliophila</i> L.	<i>H. acuminata</i> (Eckl. & Zeyh.) Steud.	1	1
		<i>H. affinis</i> Sond.	4	2

		<i>H. amplexicaulis</i> L.f.	1	1
		<i>H. arenaria</i> Sond. var. <i>arenaria</i>	1	1
		<i>H. carnosa</i> (Thunb.) Steud.	50	21
		<i>H. cornuta</i> Sond.	1	1
		<i>H. deserticola</i> Schltr.	9	6
		<i>H. digitata</i> L.f.	1	-
		<i>H. juncea</i> (P.J. Bergius) Druce	5	2
		<i>H. lactea</i> Schltr.	1	1
		<i>H. linearis</i> (Thunb.) DC. var. <i>linearis</i>	1	1
		<i>H. minima</i> (Stephens) Marais	15	8
		<i>H. refracta</i> Sond.	1	1
		<i>H. remotiflora</i> O.E.Schulz	1	1
		<i>H. rigidiuscula</i> Sond.	143	45
		<i>H. seselifolia</i> Burch. ex DC. var. <i>seselifolia</i>	1	1
		<i>H. species</i>	17	13
		<i>H. suavissima</i> Burch. ex DC.	28	15
		<i>H. trifurca</i> Burch. ex DC.	11	9
		<i>H. variabilis</i> Burch. ex DC.	2	2
Lepidieae DC.	<i>Coronopus</i> Zinn	* <i>C. didymus</i> (L.) Sm.	16	12
		* <i>C. integrifolius</i> (DC.) Spreng.	62	41
		* <i>C. species</i>	1	1
	<i>Lepidium</i> L.	<i>L. africanum</i> (Burm.f.) DC.	117	52
		* <i>L. bonariense</i> L.	79	33
		<i>L. capense</i> Thunb.	1	1
		<i>L. desertorum</i> Eckl. & Zeyh.	5	5
		<i>L. ecklonii</i> Schrad.	2	1
		<i>L. mossii</i> Thell.	3	2
		<i>L. myriocarpum</i> Sond.	1	1
		<i>L. pinnatum</i> Thunb.	2	2
		* <i>L. ruderales</i> L.	4	3
		<i>L. schinzii</i> Thell.	23	15
		<i>L. schlechteri</i> Thell.	2	2
		<i>L. species</i>	12	10
		<i>L. transvaalense</i> Marais	27	23
		<i>L. trifurcum</i> Sond.	3	3
		<i>L. virginicum</i> L.	21	11
Sisymbrieae DC.	<i>Sisymbrium</i> L.	<i>S. burchellii</i> DC. var. <i>burchellii</i>	22	16
		<i>S. capense</i> Thunb.	19	14
		<i>S. capense</i> Thunb. x <i>S. turczaninowii</i> Sond.	1	1
		* <i>S. irio</i> L.	1	1
		* <i>S. officinale</i> (L.) Scop.	5	4
		* <i>S. orientale</i> L.	20	9
		<i>S. species</i>	8	7
		<i>S. thellungii</i> O.E.Schulz	29	16
		<i>S. turczaninowii</i> Sond.	36	25

Footnote: *n* S = number of specimens collected per species; *n* Q = number of quarter degree grid cells the specimens occur in.

Appendix C

The effect of population and surface area on specimen collection data.

Both the Northern Cape and Free State were only partially included in the study. To adjust the population density to only the grids present in the study area for these two provinces, the population density of a single QDGC was multiplied by the number of grids present in the study area. Population density data was obtained from Statistics South Africa (SSA) (2014). The original data used to estimate the population of the provinces that are only partially included in the study area (Northern Cape and Free State) is indicated in Table C1.

For example, the population density of the Free State province as a whole is 2 786 757 (SSA 2014). Furthermore, the Free State comprises of 190 QDGC's in total. When the population density is divided by the number of QDGC's, the population density per QDGC is obtained (Table C1). The population density per province in the Free State is 14 667 (Table C2).

Table C1: Original data for the provinces that are only partly included in the study area: Northern Cape (right) and Free State (left).

Free State		Northern Cape	
Total grids (gr):	190	Total grids (gr):	554
Total area:	128 840.03 km ²	Total area:	372 967.17 km ²
Avg. area p.gr.:	678.11 km ²	Avg. area p.gr.:	673.23 km ²
Population:	2 786 757	Population:	1 166 680
Pop. p. grid:	14 667.14211	Pop. p. grid:	2 105.920578

Table C2: Population density per area collected for further analyses with gray areas indicating the provinces that were only partially included in the study area and the population density as determined.

Province	Total grids (gr)	Total area (km ²)	Avg. area/gr (km ²)	Population	Population/gr
Free State	125	85 144.021	681.152	1 833 392	14 667
Gauteng	26	18 018.775	693.03	12 914 817	496 723
Limpopo	176	124 446.326	707.081	5 630 464	31 991
Mpumalanga	109	75 922.64	696.538	4 229 323	38 801
North-West	154	106 696.736	692.836	3 676 274	23 872
Northern Cape	191	130 756.66	684.59	402 230	2 106
Total:	781	540 985.158	692.538	28 686 501	

The distribution of the Brassicaceae was determined by using specimen data. Unfortunately, specimen data is subjected to collectors' bias. More specimens may have been taken from areas where more people are present (high population densities). Additionally, specimen collections are dependent upon the availability of a (nearby) herbarium for storage. In areas with few or no herbaria specimens may have been donated elsewhere. Herbaria might also choose not to keep specimens due to its geographical coverage or a lack of storage space.

For these reasons, it is important to keep population density and surface area surrounding herbaria in mind (Table C1). The number of specimens collected per province compared to population sizes (SSA 2014) (Figure C1) and compared to surface area (Figure C2). These figures indicate the effects that population and surface area may have on specimen collection and consequently data available for interpretation. Population sizes were obtained from SSA (2014).

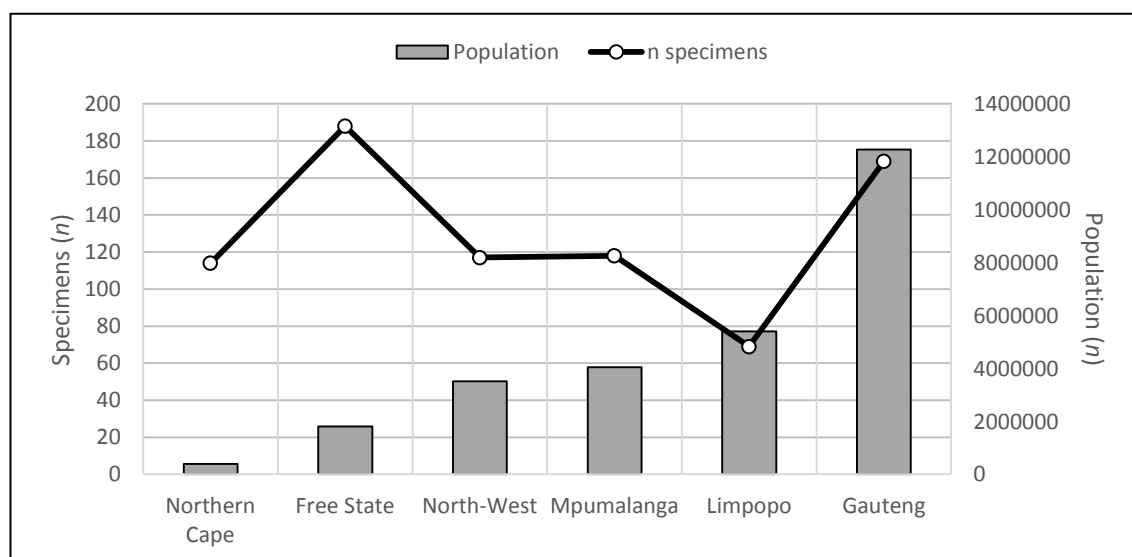


Figure C1: Specimens collected compared to population per province (SSA 2014).

The most specimens were collected in the Free State, followed by Gauteng (Figure C1). Even though the Gauteng province has the smallest surface area (Table C2, Figure C2) it contains the highest population density (Table C2, Figure C1). With the exception of Gauteng, the number of specimens collected per province does not coincide with either the population density (Figure C1) or the surface area (Figure C2) indicating that the number of specimens collected was not directly influenced by population density or surface area.

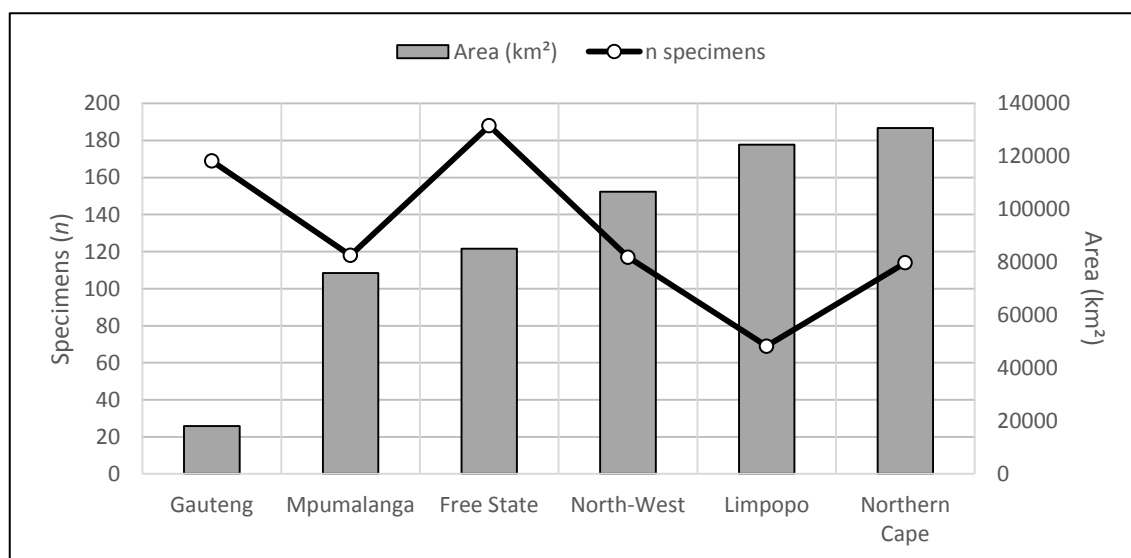


Figure C2: Specimens collected compared to surface area per province.

Table C2 indicates proportion of genera in the Brassicaceae (specimens) as a percentage of the total study area. Even though Gauteng has the smallest surface area (Table C2) 21.81% of the total specimens (Table C3) were collected in the province. 21.16% of all specimens belong to *Lepidium*, of which 5.81% were collected in Gauteng (Table C3). Table 4.5 indicates the number of species per genus in the Brassicaceae – with each species only allowed one hit per QDGC. The same proportion of species were present in both the Gauteng and Free State provinces (20.61%) even though the Free State is almost 4.73 times larger than Gauteng (Table C2).

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Table C3: Proportion of specimens ($n=1374$) per province across genera, expressed as a percentage.

Genera	Indigenous (I), Alien (A)	Provinces						Total study area
		Gauteng	Limpopo	Mpumalanga	North-West	Free State	Northern Cape	
<i>Alyssum</i> L.	A (1)	0.129	0	0	0	0	0	0.129
<i>Armoracia</i> P. Gaertn.	A (1)	0.129	0	0	0	0	0	0.129
<i>Brassica</i> L.	A (5)	0.387	0.903	1.032	0.387	0.645	0.258	3.613
<i>Camelina</i> Crantz	A (1)	0.129	0	0	0	0	0	0.129
<i>Capsella</i> Medik.	A (1)	0.387	0	0.387	0.774	0.516	0.129	2.194
<i>Cardamine</i> L.	I (1) / A (3)	0.516	0.645	1.032	0	0.129	0	2.323
<i>Coronopus</i> Zinn	A (2)	1.419	0.129	0.258	1.935	0.903	2.323	6.968
<i>Descurainia</i> Webb&Berthel.	A (1)	0.129	0	0	0	0	0	0.129
<i>Diplotaxis</i> DC.	A (2)	0.387	0.129	0	0.387	0.903	0	1.806
<i>Eruca</i> Mill.	A (1)	0.258	0	0	0	0.129	0	0.387
<i>Erucastrum</i> (DC.) C.Presl	I (3)	1.677	1.032	1.419	1.935	2.581	2.065	10.71
<i>Erysimum</i> L.	A (1)	0.129	0	0	0	0	0	0.129
<i>Heliophila</i> L.	I (20)	2.065	1.29	4	0.774	4.387	4.387	16.903
<i>Lepidium</i> L.	I (12) / A (2)	5.806	2.194	3.097	2.968	4.774	2.323	21.161
<i>Lobularia</i> Desv.	A (1)	0.258	0	0	0	0	0	0.258
<i>Matthiola</i> R.Br.	I (1) / A (1)	0.129	0.129	0	0.387	0	0.645	1.29
<i>Nasturtium</i> R.Br.	A (1)	1.548	0.516	0.258	0.516	0.645	0.129	3.613
<i>Raphanus</i> L.	A (2)	1.161	0.387	0.516	0.645	1.032	0	3.742
<i>Rapistrum</i> Crantz	A (1)	0.129	0	0	0.774	0.387	0.258	1.548
<i>Rorippa</i> Scop.	I (4)	1.677	1.161	1.548	1.806	2.323	0.774	9.29
<i>Sinapis</i> L.	A (2)	0.387	0.129	0.387	0.129	0.129	0	1.1613
<i>Sisymbrium</i> L.	I (5) / A (3)	2.968	0.129	1.161	1.677	4.645	1.419	12.00
<i>Turritis</i> L.	A (1)	0	0.129	0.129	0	0.129	0	0.387
<i>n</i> (genera) = 23	<i>n</i> (species) = 79	21.806	8.903	15.226	15.097	24.258	14.71	100

Appendix D

Species per QDGC indicating the three QDGC's that were selected during Centroid Grid profile standardization (see Chapter 3) and the number of species that were added. QDGC's highlighted in grey were outside the study area but were used when selecting species for standardization.

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
2228CB	2228CA	2228CC	2228DC	0	None	
2228CC	2228CB	2327BB	2328AA	0	None	
2228CD	2228CC	2328AA	2328BA	1	<i>Erucastum griquense</i>	x
2228DA	2228CB	2228DB	2228DC	0	None	
2228DB	2228DA	2229CA	2229CC	1	<i>Brassica rapa</i>	x
2228DC	2228DD	2228DB	2228CB	0	None	
2228DD	2229CC	2228DC	2328BA	2	<i>Brassica rapa</i>	x
					<i>Erucastum griquense</i>	x
2229AB				–	<i>Brassica rapa</i>	
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	
2229AC	2229AD	2229CA	2229AB	2	<i>Brassica elongata</i> subsp. <i>elongata</i>	
					<i>Brassica rapa</i>	x
					<i>Erucastum griquense</i>	
					<i>Lepidium africanum</i>	
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	x
2229AD	2229AC	2229BC	2229DA	3	<i>Brassica elongata</i> subsp. <i>elongata</i>	x
					<i>Erucastum griquense</i>	x
					<i>Lepidium africanum</i>	x
2229BC	2229AD	2229DA	2229DB	0	None	
2229BD	2230AC	2229DA	2229DB	1	<i>Lepidium pinnatum</i>	x
2229CA	2229AC	2229AD	2228DB	3	<i>Brassica elongata</i> subsp. <i>elongata</i>	x
					<i>Erucastum griquense</i>	x
					<i>Lepidium africanum</i>	x
2229CB	2229BC	2229DA	2229CA	0	None	
2229CC	2228DB	2229CD	2228DD	0	<i>Brassica rapa</i>	
2229CD	2229DC	2229CC	2229CA	1	<i>Brassica rapa</i>	x
2229DA	2229AD	2229BD	2229DB	0	None	
2229DB	2229DA	2229BD	2229BC	0	None	
2229DC	2229DD	2229CD	2229DA	1	<i>Nasturtium officinale</i>	x
2229DD	2229DC	2230CA	2230CC	0	<i>Nasturtium officinale</i>	
2230AC	2229BD	2230AD	2229DB	0	<i>Lepidium pinnatum</i>	
2230AD	2230AC	2230BC	2230CA	1	<i>Lepidium pinnatum</i>	x
2230BC	2230AD	2231AC	2230CB	1	<i>Brassica rapa</i>	x
2230BD	2230BC	2230DA	2230DB	0	None	
2230CA	2230CB	2229DD	2229DB	2	<i>Brassica rapa</i>	x
					<i>Nasturtium officinale</i>	x
2230CB	2230CA	2230DA	2230CC	0	<i>Brassica rapa</i>	
2230CC	2229DD	2230CB	2230CD	4	<i>Brassica juncea</i>	x
					<i>Brassica rapa</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Turritis glabra</i>	x
2230CD	2230DC	2230DA	2230CB	1	<i>Brassica juncea</i>	
					<i>Brassica rapa</i>	x
					<i>Turritis glabra</i>	
2230DA	2230CB	2230CD	2230BD	3	<i>Brassica juncea</i>	x
					<i>Brassica rapa</i>	x
					<i>Turritis glabra</i>	x
2230DB	2230DA	2230DC	2230DD	0	None	
2230DC	2230DD	2230CD	2230DB	2	<i>Brassica juncea</i>	x
					<i>Turritis glabra</i>	x
2230DD	2230DC	2231CC	2330BA	0	None	
2231CA	2231AC	2231CC	2231CD	0	None	
2231AC	2230BD	2231AD	2231CA	0	None	
2231CC	2231CA	2230DD	2331AA	0	None	
2231CD	2231CB	2331AB	2331BA	0	None	

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
2327AD				–	<i>Brassica elongata</i> subsp. <i>elongata</i>	
					<i>Rorippa fluvialis</i> var. <i>fluvialis</i>	
2327BB	2228CC	2327BC	2328AA	0	None	
2327BC	2327DA	2327AD	2327BB	3	<i>Brassica elongata</i> subsp. <i>elongata</i>	x
					<i>Erucastrium griquense</i>	x
					<i>Rorippa fluvialis</i> var. <i>fluvialis</i>	x
2327BD	2328AA	2327DB	2328AC	0	None	
2327CA	2327AD	2327CB	2327CC	2	<i>Brassica elongata</i> subsp. <i>elongata</i>	x
					<i>Lepidium bonariense</i>	
					<i>Rorippa fluvialis</i> var. <i>fluvialis</i>	x
2327CB	2327CA	2327AD	2327DA	4	<i>Brassica elongata</i> subsp. <i>elongata</i>	x
					<i>Erucastrium griquense</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Rorippa fluvialis</i> var. <i>fluvialis</i>	x
2327CC	2426BB	2327CB	2327CA	2	<i>Brassica elongata</i> subsp. <i>elongata</i>	x
					<i>Lepidium bonariense</i>	x
2327CD	2427AA	2427BA	2327CC	0	<i>Coronopus integrifolius</i>	
2327DA	2327BC	2327CB	2327AD	2	<i>Brassica elongata</i> subsp. <i>elongata</i>	x
					<i>Erucastrium griquense</i>	
					<i>Rorippa fluvialis</i> var. <i>fluvialis</i>	x
2327DB	2327BD	2327DC	2327DA	1	<i>Erucastrium griquense</i>	x
2327DC	2327DB	2427BA	2427BB	0	None	
2327DD	2327DC	2328CC	2427BB	0	None	
2328AA	2228CD	2327BD	2327AC	0	None	
2328AB	2327AC	2228CD	2328BA	1	<i>Erucastrium griquense</i>	x
2328AC	2328AA	2327AB	2327BD	0	None	
2328AD	2328BC	2328CA	2328AB	0	None	
2328BA	2228CD	2328AB	2228DD	0	<i>Erucastrium griquense</i>	
2328BB	2328BD	2329AA	2328BA	1	<i>Erucastrium griquense</i>	x
2328BC	2328AB	2328AD	2328CB	1	<i>Heliophila rigidiuscula</i>	x
2328BD	2328BB	2329AA	2328DA	0	None	
2328CA	2328AD	2328CB	2327BD	1	<i>Heliophila rigidiuscula</i>	
2328CB	2328CC	2328DA	2328CA	0	<i>Heliophila rigidiuscula</i>	
2328CC	2327DD	2427BB	2428AA	0	None	
2328CD	2328CC	2428AB	2428AA	0	None	
2328DA	2328CB	2328BD	2328DC	1	<i>Heliophila rigidiuscula</i>	x
2328DB	2329AC	2328DC	2328DD	0	None	
2328DC	2328DA	2428BA	2428BB	0	None	
2328DD	2328DB	2328DC	2329BB	0	None	
2329AA	2328BB	2328BD	2329AC	0	None	
2329AB	2329BA	2329AA	2329AD	0	None	
2329AC	2329AA	2328BD	2328DB	0	None	
2329AD	2329AC	2329BC	2329CB	1	<i>Cardamine africana</i>	x
2329BA	2329AB	2329BB	2329BC	5	<i>Brassica juncea</i>	x
					<i>Brassica rapa</i>	x
					<i>Erucastrium austroafricanum</i>	x
					<i>Raphanus sativus</i>	x
					<i>Rorippa nudiuscula</i>	x
2329BB	2329BA	2329BC	2330AA	1	<i>Brassica juncea</i>	
					<i>Brassica rapa</i>	
					<i>Erucastrium austroafricanum</i>	
					<i>Lepidium schinzii</i>	x
					<i>Raphanus sativus</i>	
					<i>Rorippa nudiuscula</i>	
2329BC	2329AD	2329BB	2329BA	5	<i>Brassica juncea</i>	x
					<i>Brassica rapa</i>	x
					<i>Erucastrium austroafricanum</i>	x
					<i>Raphanus sativus</i>	x
					<i>Rorippa nudiuscula</i>	x
2329BD	2329BB	2329BC	2330AA	6	<i>Brassica juncea</i>	x
					<i>Brassica rapa</i>	x
					<i>Erucastrium austroafricanum</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Raphanus sativus</i>	x
					<i>Rorippa nudiuscula</i>	x
2329CA	2328DD	2329CB	2329CD	5	<i>Cardamine africana</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium virginicum</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Matthiola</i> sp.	x
					<i>Rorippa nudiuscula</i>	x
2329CB	2329CA	2329DA	2329CD	4	<i>Cardamine africana</i>	
					<i>Lepidium bonariense</i>	x
					<i>Lepidium virginicum</i>	x
					<i>Matthiola</i> sp.	x
					<i>Rorippa nudiuscula</i>	x
2329CC	2329CA	2329CB	2329CD	5	<i>Cardamine africana</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium virginicum</i>	x
					<i>Matthiola</i> sp.	x
					<i>Rorippa nudiuscula</i>	x
2329CD	2329CB	2329CC	2329DC	1	<i>Cardamine africana</i>	x
					<i>Lepidium bonariense</i>	
					<i>Lepidium virginicum</i>	
					<i>Matthiola</i> sp.	
					<i>Rorippa nudiuscula</i>	
2329DA	2329CB	2329CD	2329DC	5	<i>Cardamine africana</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium virginicum</i>	x
					<i>Matthiola</i> sp.	x
					<i>Rorippa nudiuscula</i>	x
2329DB	2329BD	2329DA	2329DD	2	<i>Cardamine africana</i>	x
					<i>Nasturtium officinale</i>	x
2329DC	2329CB	2329CD	2429AB	5	<i>Cardamine africana</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium virginicum</i>	x
					<i>Matthiola</i> sp.	x
					<i>Rorippa nudiuscula</i>	x
2329DD	2329DB	2429BA	2429BB	1	<i>Cardamine africana</i>	
					<i>Nasturtium officinale</i>	
					<i>Heliophila rigidiuscula</i>	x
2330AA	2329BD	2330AC	2330AB	3	<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium schinzii</i>	
					<i>Rorippa nudiuscula</i>	x
2330AB	2330AD	2330BA	2330AA	1	<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	
					<i>Lepidium schinzii</i>	x
					<i>Rorippa nudiuscula</i>	
2330AC	2330AA	2330AB	2330CA	7	<i>Cardamine africana</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium</i> sp.	x
					<i>Lepidium virginicum</i>	x
					<i>Rorippa nudiuscula</i>	x
2330AD	2330AB	2330BC	2330BA	3	<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Rorippa nudiuscula</i>	x
2330BA	2330AB	2330AD	2330BC	3	<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Rorippa nudiuscula</i>	x
2330BB	2230DD	2330BD	2330BC	0	None	
2330BC	2330AB	2330AD	2330BA	3	<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Rorippa nudiuscula</i>	x
2330BD	2330BB	2330DB	2331AC	0	None	
2330CA	2330AC	2330CB	2330CC	2	<i>Cardamine africana</i>	
					<i>Erucastrum griquense</i>	x
					<i>Lepidium bonariense</i>	
					<i>Lepidium</i> sp.	
					<i>Lepidium virginicum</i>	
					<i>Raphanus raphanistrum</i>	x
2330CB	2330CA	2330CC	2330AC	6	<i>Cardamine africana</i>	x
					<i>Erucastrum griquense</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium</i> sp.	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Lepidium virginicum</i>	x
					<i>Raphanus raphanistrum</i>	x
2330CC	2330CA	2330CB	2329DB	3	<i>Cardamine africana</i>	
					<i>Erucastum griquense</i>	
					<i>Lepidium bonariense</i>	x
					<i>Lepidium sp.</i>	x
					<i>Lepidium virginicum</i>	x
					<i>Raphanus raphanistrum</i>	
2330CD	2430AB	2330DC	2430BA	1	<i>Nasturtium officinale</i>	x
2330DA	2330BD	2330DB	2330DC	0	None	
2330DB	2330DA	2331AA	2331AC	0	None	
2330DC	2330CD	2430AB	2430BA	1	<i>Nasturtium officinale</i>	x
2330DD	2330DC	2430BB	2431AA	1	<i>Lepidium africanum</i>	x
2331AA	2231CC	2331AC	2330BB	0	None	
2331AB	2231CD	2331AD	–	0	None	
2331AC	2331AA	2331CA	2331AD	0	None	
2331AD	2331AB	2331AC	2331CB	0	None	
2331CA	2331AC	2331CB	2331CD	1	<i>Rorippa madagascariensis</i>	x
2331CB	2331AD	2331CD	2331CA	1	<i>Rorippa madagascariensis</i>	x
2331CC	2330DD	2331CD	2331CA	1	<i>Rorippa madagascariensis</i>	x
2331CD	2331CB	2331CA	2431AB	1	<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	x
					<i>Rorippa madagascariensis</i>	
2331DA	2331AD	2331BC	2331DC	0	None	
2331DC	2331DA	2331CB	2431BA	0	None	
2420CC	2420CD	2520AA	2520AB	0	None	
2425DD	2426CC	2426BA	2425DC	0	None	
2426BB				–	<i>Brassica elongata</i> subsp. <i>elongata</i>	
2426BD	2426BC	2427AC	2426DB	0	None	
2426CC	2426CC	2526BA	2426DC	0	None	
2426CD	2426CC	2426DC	2526AB	0	None	
2426DA	2426DB	2426DD	2426CB	0	None	
2426DB	2426BD	2426DD	2426DA	0	None	
2426DC	2426CD	2426DD	2526AB	0	None	
2426DD	2426DC	2427CC	2526AB	0	None	
2427AA	2327CD	2427AB	2427AD	1	<i>Coronopus integrifolius</i>	x
2427AB	2427AA	2327CD	2427AD	1	<i>Coronopus integrifolius</i>	x
2427AC	2426BB	2426BD	2427AB	1	<i>Brassica elongata</i> subsp. <i>elongata</i>	x
2427AD	2427DA	2427AA	2427AB	0	None	
2427BA	2427BC	2427AD	2327CD	2	<i>Coronopus integrifolius</i>	x
					<i>Heliophila sp.</i>	x
2427BB	2428AA	2428AC	2328CC	0	None	
2427BC	2427BA	2427BD	2427DA	0	<i>Heliophila sp.</i>	
2427BD	2427BC	2428AC	2427DB	1	<i>Heliophila sp.</i>	x
2427CA	2427AD	2427CB	2427CC	0	None	
2427CB	2427DA	2427CD	2427CA	0	None	
2427CC	2426DD	2427CB	2427CD	0	None	
2427CD	2427CB	2427CC	2527AA	0	None	
2427DA	2427AD	2427BC	2427DC	1	<i>Heliophila sp.</i>	x
2427DB	2428CA	2428AC	2427DD	0	None	
2427DC	2527BA	2427DA	2527AB	3	<i>Erucastum austroafricanum</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	x
2427DD	2427DB	2428CA	2527BB	0	None	
2428AA	2428AC	2428AB	2427BB	0	None	
2428AB	2328CC	2428BA	2428AC	0	None	
2428AC	2428CB	2428AA	2428CA	1	<i>Erucastum austroafricanum</i>	x
2428AD	2428CB	2428BA	2428BC	1	<i>Erucastum austroafricanum</i>	x
2428BA	2428AD	2428BC	2428BB	0	None	
2428BB	2428BA	2428BC	2428BD	0	None	
2428BC	2428AD	2428BA	2428DA	3	<i>Cardamine africana</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium sp.</i>	x
2428BD	2428BC	2428DA	2429CA	4	<i>Cardamine africana</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium sp.</i>	x
					<i>Sinapis arvensis</i>	x
2428CA	2428CB	2427DB	2428AC	1	<i>Erucastum austroafricanum</i>	x
2428CB	2428AC	2428DA	2428AC	3	<i>Cardamine africana</i>	

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Erucastum austroafricanum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium</i> sp.	x
2428CC	2428CD	2428CA	2427DB	1	<i>Rorippa nudiuscula</i>	x
2428CD	2428CC	2428DC	2428CB	1	<i>Rorippa nudiuscula</i>	
					<i>Erucastum austroafricanum</i>	x
2428DA	2428CB	2428BC	2428AD	1	<i>Cardamine africana</i>	
					<i>Erucastum austroafricanum</i>	x
					<i>Lepidium africanum</i>	
					<i>Lepidium</i> sp.	
2428DB	2429CA	2428DA	2428BD	4	<i>Sinapis arvensis</i>	x
					<i>Cardamine africana</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium</i> sp.	x
2428DC	2428DB	2528AB	2428DD	1	<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
2428DD	2428DC	2429CA	2428DB	1	<i>Sinapis arvensis</i>	x
2429AA	2429AB	2429AC	2329CD	4	<i>Erucastum griquense</i>	
					<i>Heliophila carnosa</i>	
					<i>Lepidium bonariense</i>	x
					<i>Lepidium virginicum</i>	x
					<i>Matthiola</i> sp.	x
					<i>Nasturtium officinale</i>	
					<i>Rorippa nudiuscula</i>	x
2429AB	2329CD	2429BA	2429AA	8	<i>Erucastum griquense</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium virginicum</i>	x
					<i>Matthiola</i> sp.	x
					<i>Nasturtium officinale</i>	x
					<i>Rorippa nudiuscula</i>	x
2429AC	2429CA	2429AD	2429BD	1	<i>Sinapis arvensis</i>	x
2429AD	2429AC	2429CA	2429CB	1	<i>Sinapis arvensis</i>	x
2429BA	2429AB	2329DD	2329DC	2	<i>Cardamine africana</i>	x
					<i>Heliophila rigidiuscula</i>	
					<i>Nasturtium officinale</i>	x
2429BB	2329DD	2430AA	2429BA	7	<i>Cardamine africana</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium virginicum</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Sisymbrium officinale</i>	x
2429BC	2429AD	2429BD	2429DB	1	<i>Diplotaxis muralis</i>	x
2429BD	2430AC	2429DB	2430CA	1	<i>Diplotaxis muralis</i>	
					<i>Heliophila rigidiuscula</i>	x
2429CA	2428DD	2429AC	2429CC	0	<i>Sinapis arvensis</i>	
2429CB	2429CA	2429CD	2429CC	2	<i>Sinapis arvensis</i>	x
2429CC	2429CD	2429CA	2429CB	2	<i>Lepidium bonariense</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Sinapis arvensis</i>	x
2429CD	2429DA	2429CC	2429DC	0	<i>Lepidium bonariense</i>	
2429DA	2429CD	2429DC	2429BC	1	<i>Lepidium bonariense</i>	x
2429DB	2429DB	2430CC	2430CA	3	<i>Diplotaxis muralis</i>	x
					<i>Heliophila acuminata</i>	x
					<i>Heliophila rigidiuscula</i>	x
2429DC	2429CD	2429DA	2429DD	1	<i>Lepidium bonariense</i>	x
2429DD	2429DC	2429DB	2529BB	2	<i>Erucastum austroafricanum</i>	x
					<i>Heliophila rigidiuscula</i>	x
2430AA	2429BB	2430AD	2329DD	2	<i>Cardamine africana</i>	x
					<i>Heliophila rigidiuscula</i>	
					<i>Lepidium africanum</i>	
					<i>Lepidium virginicum</i>	
					<i>Nasturtium officinale</i>	x
					<i>Raphanus raphanistrum</i>	
					<i>Sisymbrium officinale</i>	
2430AB	2430BC	2330CD	2430AD	1	<i>Heliophila rigidiuscula</i>	x
					<i>Nasturtium officinale</i>	

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
2430AC	2430CA	2430CB	2430AA	5	<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium virginicum</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Sisymbrium officinale</i>	x
2430AD	2430AA	2430DA	2430AB	5	<i>Heliophila rigidiuscula</i>	
					<i>Lepidium africanum</i>	x
					<i>Lepidium virginicum</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Sisymbrium officinale</i>	x
2430BA	2330CD	2430AB	2430BB	1	<i>Nasturtium officinale</i>	x
2430BB	2430BA	2330DC	2431AA	1	<i>Lepidium africanum</i>	x
2430BC	2430AB	2430BD	2430BA	2	<i>Nasturtium officinale</i>	x
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	x
2430BD	2430BC	2430BA	2431AC	1	<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	
2430CA	2430AC	2430CC	2430CB	1	<i>Heliophila acuminata</i>	x
					<i>Heliophila rigidiuscula</i>	
2430CB	2430AC	2430CA	2430AD	1	<i>Heliophila rigidiuscula</i>	x
2430CC	2430CA	2529BB	2530AA	2	<i>Erucastrum austroafricanum</i>	x
					<i>Heliophila acuminata</i>	
					<i>Heliophila rigidiuscula</i>	x
2430CD	2430DA	2430DC	2430CB	5	<i>Cardamine africana</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Heliophila trifurca</i>	x
					<i>Lepidium virginicum</i>	x
2430DA	2430CD	2430DC	2430CB	5	<i>Cardamine africana</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Heliophila trifurca</i>	x
					<i>Lepidium virginicum</i>	x
2430DB	2430DD	2431CC	2430BC	0	<i>Cardamine africana</i>	
					<i>Heliophila rigidiuscula</i>	
2430DC	2430DA	2430CD	2530AB	5	<i>Cardamine africana</i>	
					<i>Brassica rapa</i>	x
					<i>Erucastrum austroafricanum</i>	
					<i>Erucastrum griquense</i>	x
					<i>Heliophila rigidiuscula</i>	
					<i>Heliophila trifurca</i>	
					<i>Lepidium africanum</i>	x
					<i>Lepidium virginicum</i>	
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa nudiuscula</i>	x
2430DD	2430DB	2530BB	2431CC	2	<i>Cardamine africana</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Lepidium africanum</i>	x
					<i>Nasturtium officinale</i>	x
2431AA	2431AB	2430BB	2431AD	1	<i>Lepidium africanum</i>	
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	x
2431AB	2331CD	2331CC	2431AA	2	<i>Lepidium africanum</i>	x
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	
					<i>Rorippa madagascariensis</i>	x
2431AC	2431AD	2431BB	2431CB	2	<i>Brassica</i> sp.	x
					<i>Lepidium africanum</i>	x
2431AD	2431AC	2431AA	2431BC	0	<i>Lepidium africanum</i>	
2431BA	2331CD	2431AB	2331DC	2	<i>Rorippa madagascariensis</i>	x
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	x
2431BB	2431BD	2431BA	2432AC	1	<i>Rorippa madagascariensis</i>	x
2431BC	2431AD	2431DA	2431BA	1	<i>Lepidium africanum</i>	x
2431BD	2431BB	2431DB	2431BA	0	<i>Rorippa madagascariensis</i>	
2431CA	2431CC	2431CB	2431AC	2	<i>Brassica</i> sp.	x
					<i>Lepidium africanum</i>	x
2431CB	2431AC	2431CD	2431CA	0	<i>Brassica</i> sp.	
					<i>Lepidium africanum</i>	
2431CC	2431CA	2431CD	2431CB	2	<i>Brassica</i> sp.	x
					<i>Lepidium africanum</i>	x
2431CD	2431CB	2431CA	2531BA	2	<i>Brassica</i> sp.	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Lepidium africanum</i>	x
2431DA	2431BC	2431DD	2431DC	0	None	
2431DB	2431BD	2431DD	2432CA	1	<i>Rorippa madagascariensis</i>	x
2431DC	2431DA	2431CD	2431CB	2	<i>Brassica</i> sp.	x
					<i>Lepidium africanum</i>	x
2431DD	2431DB	2531BB	2532CC	0	None	
2520AA	2420CC	2520AB	2520AC	0	None	
2520AB	2420CC	2520AA	2520AD	0	None	
2520AC	2520AD	2520CA	2520CB	2	<i>Heliophila deserticola</i>	x
					<i>Heliophila suavisissima</i>	x
2520AD	2520AC	2520CB	2520DA	2	<i>Heliophila deserticola</i>	x
					<i>Heliophila suavisissima</i>	x
2520CA	2520AC	2520CB	2520CD	2	<i>Heliophila deserticola</i>	x
					<i>Heliophila suavisissima</i>	x
2520CB	2520AC	2520CA	2520CD	0	<i>Heliophila deserticola</i>	
					<i>Heliophila suavisissima</i>	
2520CC	2520CD	2620AA	2620AB	0	<i>Coronopus integrifolius</i>	
2520CD	2520CA	2520CB	2520DC	2	<i>Heliophila deserticola</i>	x
					<i>Heliophila suavisissima</i>	x
2520DA	2520DC	2520CD	2520DD	0	None	
2520DC	2520CD	2520CB	2520DA	2	<i>Heliophila deserticola</i>	x
					<i>Heliophila suavisissima</i>	x
2522DB	2522DD	2522CD	2523CA	0	None	
2522DD	2522DB	2622BA	2523CC	0	None	
2523AC	2523AD	2523CA	2522DB	0	None	
2523AD	2523AC	2523BC	2523DA	0	None	
2523CA	2523AC	2522DD	2523CC	0	None	
2523CB	2523CA	2523AD	2523AD	0	None	
2523CC	2522DD	2523CA	2523CD	0	None	
2523CD	2523CA	2623AA	2523DC	0	None	
2523DA	2523CB	2523DB	2523BC	1	<i>Coronopus integrifolius</i>	x
2523DB	2523CC	2523DA	2523DD	0	<i>Coronopus integrifolius</i>	
2523DC	2523CD	2523DD	2623AA	0	None	
2523DD	2523DC	2523DB	2523DA	1	<i>Coronopus integrifolius</i>	x
2524CC	2524CA	2523DB	2624AA	1	<i>Coronopus integrifolius</i>	x
2524CD	2624AA	2524DC	2624BA	0	<i>Coronopus integrifolius</i>	
2524DC	2524CD	2624AB	2624BA	1	<i>Coronopus integrifolius</i>	x
2524DD	2525CC	2524DC	2624BA	1	<i>Brassica elongata</i> subsp. <i>elongata</i>	x
2525BB	2425DD	2426CC	2526AA	0	None	
2525BD	2525BC	2526AC	2526CA	2	<i>Lepidium africanum</i>	x
					<i>Rorippa fluvialis</i> var. <i>fluvialis</i>	x
2525CC	2524DD	2525CD	2625AA	1	<i>Brassica elongata</i> subsp. <i>elongata</i>	
					<i>Sisymbrium turczaninowii</i>	x
2525CD	2625AB	2525CB	2525CC	1	<i>Brassica elongata</i> subsp. <i>elongata</i>	x
					<i>Sisymbrium turczaninowii</i>	
2525DA	2525DC	2525CD	2525CB	2	<i>Coronopus integrifolius</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2525DB	2526CC	2525DD	2525DA	4	<i>Erucastrium strigosum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2525DC	2525DA	2625BB	2625BA	0	<i>Coronopus integrifolius</i>	
2525DD	2525DB	2526CC	2626AA	6	<i>Erucastrium griquense</i>	x
					<i>Erucastrium strigosum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium</i> sp.	x
					<i>Raphanus raphanistrum</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2526AA	2525BB	2526AD	2526AB	1	<i>Lepidium africanum</i>	x
2526AB	2526AA	2526BA	2426CD	0	None	
2526AC	2525BD	2526AD	2526CA	2	<i>Lepidium africanum</i>	x
					<i>Rorippa fluvialis</i> var. <i>fluvialis</i>	x
2526AD	2526AC	2526CB	2526BC	0	<i>Lepidium africanum</i>	
2526BA	2526AB	2526BB	2426DC	0	None	
2526BB	2526BA	2426DD	2527AA	0	None	
2526BC	2526AD	2526BD	2526DA	1	<i>Lepidium africanum</i>	x
2526BD	2526BC	2526DB	2527AC	0	None	
2526CA	2526CB	2525BD	2526AC	0	<i>Lepidium africanum</i>	

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	
2526CB	2526CA	2526AD	2526DA	2	<i>Lepidium africanum</i>	x
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	x
2526CC	2525DD	2526CD	2626AA	2	<i>Erucastrum griquense</i>	x
					<i>Erucastrum strigosum</i>	
					<i>Lepidium africanum</i>	
					<i>Lepidium</i> sp.	x
					<i>Raphanus raphanistrum</i>	
					<i>Sisymbrium turczaninowii</i>	
2526CD	2626BA	2526CC	2626AA	5	<i>Erucastrum austroafricanum</i>	x
					<i>Erucastrum griquense</i>	x
					<i>Lepidium africanum</i>	
					<i>Lepidium</i> sp.	x
					<i>Raphanus raphanistrum</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2526DA	2526CB	2526BC	2526DB	0	None	
2526DB	2526BD	2526DA	2526BC	0	None	
2526DC	2526DD	2626BB	2626BA	0	None	
2526DD	2526DC	2527CC	2627AA	3	<i>Coronopus integrifolius</i>	x
					<i>Erucastrum strigosum</i>	x
					<i>Rorippa nudiuscula</i>	x
2527AA	2526BB	2427CD	2527AC	0	None	
2527AB	2527BC	2527BA	2527BD	3	<i>Erucastrum austroafricanum</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	x
2527AC	2526BD	2527CA	2526BB	2	<i>Heliophila rigidiuscula</i>	x
					<i>Heliophila</i> sp.	x
2527AD	2527AB	2527BC	2527AC	0	None	
2527BA	2527BB	2527AB	2427DD	0	<i>Erucastrum austroafricanum</i>	
					<i>Rapistrum rugosum</i>	
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	
2527BB	2527BA	2427DD	2427DC	3	<i>Erucastrum austroafricanum</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	x
2527BC	2527AD	2527BD	2527AB	1	<i>Lepidium</i> sp.	x
2527BD	2528AC	2527BB	2527BC	1	<i>Coronopus didymus</i>	x
					<i>Lepidium</i> sp.	
2527CA	2526DB	2527AC	2527CD	1	<i>Heliophila rigidiuscula</i>	
					<i>Heliophila</i> sp.	
					<i>Lepidium virginicum</i>	x
2527CB	2527DA	2527CA	2527CD	3	<i>Rapistrum rugosum</i>	
2527CC	2526DD	2527CA	2527CD	3	<i>Heliophila rigidiuscula</i>	x
					<i>Heliophila</i> sp.	x
					<i>Lepidium virginicum</i>	x
2527CD	2527DC	2527CC	2527CA	4	<i>Lepidium virginicum</i>	
					<i>Cardamine africana</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Heliophila</i> sp.	x
					<i>Lepidium africanum</i>	x
2527DA	2527CB	2527DB	2527BD	4	<i>Erucastrum strigosum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium</i> sp.	x
					<i>Rapistrum rugosum</i>	x
2527DB	2527DC	2527DA	2528CA	19	<i>Brassica rapa</i>	x
					<i>Camelina rumelica</i>	x
					<i>Capsella bursa-pastoris</i>	x
					<i>Cardamine africana</i>	x
					<i>Coronopus didymus</i>	x
					<i>Diplotaxis muralis</i>	x
					<i>Eruca sativa</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Erucastrum strigosum</i>	
					<i>Heliophila juncea</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	x
					<i>Lepidium virginicum</i>	x
					<i>Nasturtium officinale</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Raphanus raphanistrum</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sinapis arvensis</i>	x
					<i>Sisymbrium thellungii</i>	x
2527DC	2527CD	2527DD	2527DB	6	<i>Cardamine africana</i>	
					<i>Diplotaxis muralis</i>	x
					<i>Erucastum strigosum</i>	x
					<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Sisymbrium officinale</i>	x
2527DD	2628AA	2528CC	2627BA	28	<i>Alyssum minutum</i>	x
					<i>Armoracia rusticana</i>	x
					<i>Brassica rapa</i>	x
					<i>Capsella bursa-pastoris</i>	x
					<i>Cardamine hirsuta</i>	x
					<i>Coronopus didymus</i>	x
					<i>Coronopus integrifolius</i>	x
					<i>Diplotaxis muralis</i>	
					<i>Erucastum austroafricanum</i>	x
					<i>Erucastum strigosum</i>	x
					<i>Erysimum cheiri</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila juncea</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	
					<i>Lepidium capense</i>	x
					<i>Lepidium mossii</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium transvaalense</i>	
					<i>Lepidium virginicum</i>	x
					<i>Lobularia maritima</i>	x
					<i>Matthiola incana</i>	x
					<i>Nasturtium officinale</i>	
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sinapis alba</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium officinale</i>	
					<i>Sisymbrium orientale</i>	x
					<i>Sisymbrium</i> sp.	x
					<i>Sisymbrium thellungii</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2528AA	2528AB	2428CC	2527BB	1	<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	x
					<i>Sinapis alba</i>	
2528AB	2428DC	2528BA	2528AA	1	<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	
					<i>Sinapis alba</i>	x
2528AC	2528CA	2528AD	2527BD	18	<i>Brassica rapa</i>	x
					<i>Camelina rumelica</i>	x
					<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus didymus</i>	
					<i>Diplotaxis muralis</i>	x
					<i>Eruca sativa</i>	x
					<i>Erucastum austroafricanum</i>	x
					<i>Erucastum strigosum</i>	x
					<i>Heliophila juncea</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium</i> sp.	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Rorippa nudiuscula</i>	x
					<i>Sinapis arvensis</i>	x
					<i>Sisymbrium thellungii</i>	x
2528AD	2528AC	2528CB	2528BC	8	<i>Cardamine hirsuta</i>	x
					<i>Coronopus didymus</i>	
					<i>Erucastrum austroafricanum</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium virginicum</i>	
					<i>Nasturtium officinale</i>	
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	x
					<i>Sinapis arvensis</i>	
2528BA	2528AB	2428DD	2428DC	1	<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	x
2528BB	2528BA	2528BC	2529AA	1	<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	x
2528BC	2528AD	2528DA	2528BD	5	<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	
					<i>Coronopus didymus</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium virginicum</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Sinapis arvensis</i>	x
2528BD	2528BB	2529AA	2528BC	1	<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	x
2528CA	2528CB	2527DB	2528CD	6	<i>Brassica rapa</i>	
					<i>Camelina rumelica</i>	
					<i>Capsella bursa-pastoris</i>	
					<i>Cardamine hirsuta</i>	x
					<i>Coronopus didymus</i>	
					<i>Coronopus integrifolius</i>	x
					<i>Diplotaxis muralis</i>	
					<i>Eruca sativa</i>	
					<i>Erucastrum austroafricanum</i>	
					<i>Erucastrum strigosum</i>	
					<i>Heliophila juncea</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	
					<i>Lepidium schinzii</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Lepidium virginicum</i>	
					<i>Nasturtium officinale</i>	
					<i>Raphanus raphanistrum</i>	
					<i>Raphanus sativus</i>	x
					<i>Rapistrum rugosum</i>	
					<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	
					<i>Rorippa nudiuscula</i>	
					<i>Sinapis arvensis</i>	
					<i>Sisymbrium thellungii</i>	
					<i>Sisymbrium turczaninowii</i>	x
2528CB	2528CA	2528CD	2528AD	17	<i>Cardamine hirsuta</i>	
					<i>Brassica rapa</i>	x
					<i>Camelina rumelica</i>	x
					<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus didymus</i>	
					<i>Coronopus integrifolius</i>	x
					<i>Diplotaxis muralis</i>	x
					<i>Eruca sativa</i>	x
					<i>Erucastrum austroafricanum</i>	
					<i>Erucastrum strigosum</i>	x
					<i>Heliophila juncea</i>	x
					<i>Heliophila rigidiuscula</i>	
					<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	
					<i>Lepidium schinzii</i>	
					<i>Lepidium transvaalense</i>	x
					<i>Lepidium virginicum</i>	
					<i>Nasturtium officinale</i>	

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Raphanus raphanistrum</i>	
					<i>Raphanus sativus</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sinapis arvensis</i>	x
					<i>Sisymbrium thellungii</i>	x
					<i>Sisymbrium thellungii</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2528CC	2527DD	2528CD	2628AA	20	<i>Capsella bursa-pastoris</i>	
					<i>Alyssum minutum</i>	x
					<i>Armoracia rusticana</i>	x
					<i>Brassica rapa</i>	x
					<i>Cardamine hirsuta</i>	x
					<i>Coronopus didymus</i>	
					<i>Coronopus integrifolius</i>	x
					<i>Diplotaxis muralis</i>	x
					<i>Erucastrum austroafricanum</i>	
					<i>Erucastrum strigosum</i>	x
					<i>Erysimum cheiri</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila juncea</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	
					<i>Lepidium capense</i>	
					<i>Lepidium mossii</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium transvaalense</i>	
					<i>Lepidium virginicum</i>	x
					<i>Lobularia maritima</i>	
					<i>Matthiola incana</i>	x
					<i>Nasturtium officinale</i>	
					<i>Raphanus raphanistrum</i>	
					<i>Raphanus sativus</i>	x
					<i>Rorippa nudiuscula</i>	
					<i>Sinapis alba</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium officinale</i>	x
					<i>Sisymbrium orientale</i>	
					<i>Sisymbrium thellungii</i>	
					<i>Sisymbrium turczaninowii</i>	x
2528CD	2528CC	2528CB	2628AA	25	<i>Coronopus integrifolius</i>	
					<i>Alyssum minutum</i>	x
					<i>Armoracia rusticana</i>	x
					<i>Brassica rapa</i>	x
					<i>Capsella bursa-pastoris</i>	x
					<i>Cardamine hirsuta</i>	x
					<i>Coronopus didymus</i>	x
					<i>Diplotaxis muralis</i>	x
					<i>Erucastrum austroafricanum</i>	
					<i>Erucastrum strigosum</i>	x
					<i>Erysimum cheiri</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila juncea</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	
					<i>Lepidium capense</i>	x
					<i>Lepidium mossii</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium transvaalense</i>	
					<i>Lepidium virginicum</i>	x
					<i>Lobularia maritima</i>	x
					<i>Matthiola incana</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Raphanus sativus</i>	

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Rorippa nudiuscula</i>	x
					<i>Sinapis alba</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium orientale</i>	x
					<i>Sisymbrium thellungii</i>	
					<i>Sisymbrium turczaninowii</i>	
2528DA	2528DB	2528CB	2528BD	9	<i>Cardamine hirsuta</i>	x
					<i>Coronopus didymus</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium virginicum</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Raphanus raphanistrum</i>	x
2528DB	2528DA	2529CA	2528DD	2	<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
2528DC	2528DD	2528DB	2628BA	2	<i>Heliophila rigidiuscula</i>	
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Nasturtium officinale</i>	
2528DD	2528DB	2529CC	2529CA	2	<i>Coronopus didymus</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	
2529AA	2529AB	2429CD	2528BB	1	<i>Lepidium bonariense</i>	x
2529AB	2529AD	2529AA	2429CD	3	<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	x
2529AC	2528BB	2528BD	2529AA	0	None	
2529AD	2529AB	2529AA	2529BC	0	<i>Heliophila rigidiuscula</i>	
					<i>Lepidium bonariense</i>	
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	
2529BA	2429DC	2529BC	2429DD	0	None	
2529BB	2530AA	2529BD	2429DD	1	<i>Erucastrum austroafricanum</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Lepidium bonariense</i>	x
2529BC	2529BA	2529CB	2529DA	2	<i>Heliophila rigidiuscula</i>	x
					<i>Heliophila trifurca</i>	x
2529BD	2529BB	2529DB	2530AA	2	<i>Erucastrum austroafricanum</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium bonariense</i>	
2529CA	2528DB	2529AC	2529CB	2	<i>Heliophila rigidiuscula</i>	x
					<i>Heliophila trifurca</i>	x
2529CB	2529DA	2529CA	2529BC	0	<i>Heliophila rigidiuscula</i>	
					<i>Heliophila trifurca</i>	
2529CC	2529CD	2528DD	2628BB	4	<i>Coronopus didymus</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Sisymbrium thellungii</i>	x
2529CD	2529DC	2529CC	2628BB	1	<i>Heliophila rigidiuscula</i>	
					<i>Coronopus didymus</i>	x
					<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	
					<i>Lepidium transvaalense</i>	
					<i>Sisymbrium thellungii</i>	
2529DA	2529CB	2529CD	2529BC	6	<i>Heliophila rigidiuscula</i>	x
					<i>Heliophila trifurca</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Sisymbrium thellungii</i>	x
2529DB	2529BD	2529DA	2530AC	3	<i>Heliophila rigidiuscula</i>	
					<i>Heliophila carnosa</i>	x
					<i>Heliophila</i> sp.	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Lepidium bonariense</i>	x
2529DC	2529CD	2529DA	2629BA	6	<i>Erucastrum austroafricanum</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Sisymbrium thellungii</i>	x
2529DD	2629BA	2629BB	2630AA	3	<i>Erucastrum austroafricanum</i>	x
					<i>Heliophila carnososa</i>	x
					<i>Heliophila rigidiuscula</i>	x
2530AA	2529BB	2430CC	2529BD	2	<i>Heliophila rigidiuscula</i>	
					<i>Erucastrum austroafricanum</i>	x
					<i>Heliophila acuminata</i>	x
					<i>Lepidium bonariense</i>	x
2530AB	2530AD	2430DC	2530BA	6	<i>Brassica rapa</i>	
					<i>Capsella bursa-pastoris</i>	x
					<i>Cardamine africana</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Erucastrum griquense</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Heliophila sp.</i>	x
					<i>Heliophila trifurca</i>	x
					<i>Lepidium africanum</i>	
					<i>Lepidium virginicum</i>	x
					<i>Raphanus raphanistrum</i>	
					<i>Rorippa nudiuscula</i>	
2530AC	2530CA	2530CB	2530AB	8	<i>Heliophila carnososa</i>	
					<i>Brassica rapa</i>	x
					<i>Capsella bursa-pastoris</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Erucastrum griquense</i>	x
					<i>Heliophila rigidiuscula</i>	
					<i>Heliophila sp.</i>	
					<i>Lepidium africanum</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa nudiuscula</i>	x
2530AD	2530AB	2530CB	2530BA	7	<i>Brassica rapa</i>	
					<i>Capsella bursa-pastoris</i>	
					<i>Cardamine africana</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Erucastrum griquense</i>	x
					<i>Heliophila rigidiuscula</i>	
					<i>Heliophila sp.</i>	
					<i>Lepidium africanum</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa nudiuscula</i>	x
2530BA	2430DC	2530AB	2530AD	11	<i>Brassica rapa</i>	x
					<i>Capsella bursa-pastoris</i>	x
					<i>Cardamine africana</i>	
					<i>Erucastrum austroafricanum</i>	x
					<i>Erucastrum griquense</i>	x
					<i>Heliophila rigidiuscula</i>	
					<i>Heliophila rigidiuscula</i>	x
					<i>Heliophila sp.</i>	x
					<i>Heliophila trifurca</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium virginicum</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa nudiuscula</i>	x
2530BB	2430DD	2530DB	2530BC	8	<i>Cardamine africana</i>	
					<i>Brassica nigra</i>	x
					<i>Brassica rapa</i>	x
					<i>Cardamine hirsuta</i>	x
					<i>Coronopus didymus</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium africanum</i>	

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Lepidium bonariense</i>	x
					<i>Nasturtium officinale</i>	
					<i>Raphanus raphanistrum</i>	x
					<i>Sisymbrium thellungii</i>	x
2530BC	2530DA	2530CB	2530BA	5	<i>Brassica rapa</i>	
					<i>Cardamine africana</i>	x
					<i>Cardamine hirsuta</i>	
					<i>Erucastrum austroafricanum</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium thellungii</i>	
2530BD	2530BB	2530DB	2530BC	5	<i>Brassica nigra</i>	
					<i>Brassica rapa</i>	x
					<i>Cardamine africana</i>	x
					<i>Cardamine hirsuta</i>	x
					<i>Coronopus didymus</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	
					<i>Nasturtium officinale</i>	x
					<i>Raphanus raphanistrum</i>	
					<i>Sisymbrium thellungii</i>	x
2530CA	2530AC	2530CB	2530CC	4	<i>Brassica rapa</i>	
					<i>Capsella bursa-pastoris</i>	
					<i>Erucastrum austroafricanum</i>	
					<i>Heliophila carnosa</i>	x
					<i>Heliophila rigidiuscula</i>	
					<i>Heliophila sp.</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Raphanus raphanistrum</i>	
					<i>Rorippa nudiuscula</i>	x
2530CB	2530AC	2530CA	2530DA	5	<i>Brassica rapa</i>	x
					<i>Capsella bursa-pastoris</i>	x
					<i>Erucastrum austroafricanum</i>	
					<i>Heliophila carnosa</i>	x
					<i>Heliophila rigidiuscula</i>	
					<i>Heliophila sp.</i>	x
					<i>Lepidium transvaalense</i>	
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa nudiuscula</i>	
2530CC	2630AB	2530CD	2630AA	2	<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium sp.</i>	x
2530CD	2530CC	2630AB	2630BA	1	<i>Lepidium sp.</i>	x
2530DA	2530BC	2530AD	2530BD	9	<i>Brassica rapa</i>	x
					<i>Capsella bursa-pastoris</i>	x
					<i>Cardamine hirsuta</i>	x
					<i>Coronopus didymus</i>	x
					<i>Heliophila rigidiuscula</i>	
					<i>Heliophila sp.</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Sisymbrium thellungii</i>	x
2530DB	2530BD	2530BC	2530DA	9	<i>Brassica nigra</i>	x
					<i>Brassica rapa</i>	x
					<i>Cardamine hirsuta</i>	x
					<i>Coronopus didymus</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Sisymbrium thellungii</i>	x
2530DC	2630BB	2630BA	2631AA	2	<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium virginicum</i>	x
2530DD	2531CC	2531CA	2631AA	5	<i>Cardamine africana</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila rigidiuscula</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Lepidium africanum</i>	x
					<i>Sinapis arvensis</i>	x
2531AA	2531AC	2431CC	2530BD	6	<i>Brassica nigra</i>	x
					<i>Coronopus didymus</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Raphanus raphanistrum</i>	x
2531AB	2531AC	2531AD	2431CD	0	<i>Lepidium bonariense</i>	
2531AC	2531AD	2531AA	2531CA	1	<i>Sinapis arvensis</i>	x
2531AD	2531AC	2531CA	2531AB	2	<i>Lepidium bonariense</i>	x
					<i>Sinapis arvensis</i>	x
2531BA	2431CD	2431DC	2531BC	0	None	
2531BB	2431DD	2531BD	2431DC	0	None	
2531BC	2531BA	2531DA	2531AB	1	<i>Lepidium bonariense</i>	x
2531BD	2531BB	2531DB	2531CA	0	None	
2531CA	2531AC	2531AD	2530DB	0	<i>Sinapis arvensis</i>	
2531CB	2531CA	2531CC	2531DA	5	<i>Cardamine africana</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium africanum</i>	x
					<i>Sinapis arvensis</i>	x
2531CC	2530DD	2531CB	2531CA	1	<i>Cardamine africana</i>	
					<i>Heliophila carnosa</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Lepidium africanum</i>	
					<i>Sinapis arvensis</i>	x
2531DA	2531CB	2531BC	2531DC	0	None	
2531DB	2531BD	2531DD	2531DA	0	None	
2531DD	2531DB	2631BB	2532CC	0	None	
2620AA	2520CC	2620AB	2620AD	1	<i>Coronopus integrifolius</i>	x
2620AB	2620AA	2520CC	2620BC	1	<i>Coronopus integrifolius</i>	x
2620AC	2620CB	2620CA	2620AD	0	None	
2620AD	2620AA	2620CB	2620BC	1	<i>Coronopus integrifolius</i>	x
2620BA	2520CD	2520DC	2620AB	0	None	
2620BC	2620AB	2620AD	2620BA	0	<i>Coronopus integrifolius</i>	x
2620CA	2620CB	2620CD	2620CC	0	None	
2620CB	2620AC	2620CD	2620AD	0	None	
2620CC	2620CA	2620CD	2720AA	0	None	
2620CD	2620CA	2720AA	2720AB	0	None	
2620DC	2620DD	2620DA	2620CB	0	None	
2621CC	2621CD	2621DD	2721AA	0	None	
2621CD	2621CC	2621DC	2721AA	0	None	
2621DC	2621CD	2621DD	2621DB	0	None	
2621DD	2621DC	2621DA	2621DB	0	None	
2622AD	2622AA	2621AB	2621AC	0	None	
2622BB	2522DD	2523CC	2622BD	0	None	
2622BC	2622BB	2622BD	2622BA	0	None	
2622BD	2622BB	2622BC	2623AC	0	None	
2622CA	2622AD	2622CB	2622AC	0	None	
2622CB	2622DC	2622CD	2622CC	0	None	
2622CC	2622CA	2622CB	2622CD	0	None	
2622CD	2622CC	2622CB	2622DC	0	None	
2622DA	2622BC	2622DB	2622DD	0	None	
2622DB	2622DA	2622BD	2623CA	0	None	
2622DC	2622CD	2622DD	2622CB	0	None	
2622DD	2622DA	2622DC	2722BB	0	None	
2623AA	2623AC	2523CD	2523CC	0	None	
2623AB	2623AA	2523CC	2523CD	0	None	
2623AC	2623AA	2622BD	2623AB	0	None	
2623AD	2623AB	2623AA	2623CB	0	None	
2623BA	2623AB	2623AD	2623BB	0	None	
2623BB	2623BA	2623BC	2623BD	0	None	
2623BC	2623AB	2623BA	2623DA	0	None	
2623BD	2623BC	2623DA	2623BA	0	None	
2623CA	2623CB	2623AD	2622BD	0	None	
2623CB	2623CA	2623DA	2623AD	0	None	
2623CC	2723AA	2623CD	2623CA	1	<i>Erucastrum griquense</i>	

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
2623CD	2623CC	2623DC	2723AB	0	None	
2623DA	2623CB	2623BC	2623BD	0	None	
2623DB	2624AC	2623DD	2624CA	0	<i>Coronopus integrifolius</i>	x
					<i>Raphanus raphanistrum</i>	
					<i>Rapistrum rugosum</i>	
2623DC	2623CD	2623DA	2623CB	0	None	
2623DD	2623DB	2623DA	2624CA	3	<i>Coronopus integrifolius</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rapistrum rugosum</i>	x
2624AA	2524CC	2624AC	2624AD	2	<i>Coronopus integrifolius</i>	x
					<i>Diplotaxis muralis</i>	x
2624AB	2624AD	2524CD	2624BA	2	<i>Coronopus integrifolius</i>	x
					<i>Diplotaxis muralis</i>	x
2624AC	2623DB	2624AA	2624CA	3	<i>Coronopus integrifolius</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rapistrum rugosum</i>	x
2624AD	2624AB	2624BC	2624CA	0	<i>Coronopus integrifolius</i>	
					<i>Diplotaxis muralis</i>	
2624BA	2624AB	2524DC	2624AD	2	<i>Coronopus integrifolius</i>	x
					<i>Diplotaxis muralis</i>	x
2624BB	2624BA	2524DD	2525CC	1	<i>Brassica elongata</i> subsp. <i>elongata</i>	x
2624BC	2624BA	2624AD	2624BD	2	<i>Coronopus integrifolius</i>	x
					<i>Diplotaxis muralis</i>	x
2624BD	2624BC	2624BB	2624BA	0	None	
2624CA	2623DD	2624CB	2624CC	0	None	
2624CB	2624CC	2624CA	2624BC	0	None	
2624CC	2624CB	2624CA	2624CD	0	None	
2624CD	2724AB	2624CC	2624DA	3	<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus didymus</i>	x
					<i>Sisymbrium capense</i>	x
2624DA	2624CB	2624BD	2624DB	0	None	
2624DB	2624DA	2624CA	2624BD	0	None	
2624DC	2624DD	2724BA	2624CD	0	<i>Coronopus integrifolius</i>	
					<i>Heliophila</i> sp.	
					<i>Sisymbrium turczaninowii</i>	
2624DD	2624DC	2724BA	2724BB	3	<i>Coronopus integrifolius</i>	x
					<i>Heliophila</i> sp.	x
					<i>Sisymbrium turczaninowii</i>	x
2625AA	2525CC	2624BB	2524DD	1	<i>Brassica elongata</i> subsp. <i>elongata</i>	x
2625AB	2625AD	2525CD	2525DC	2	<i>Sisymbrium turczaninowii</i>	x
					<i>Coronopus integrifolius</i>	x
2625AC	2625AA	2624DB	2625AB	0	None	
2625AD	2625AB	2625BA	2625BC	0	None	
2625BA	2625AB	2625AD	2525DC	1	<i>Coronopus integrifolius</i>	x
2625BB	2525DD	2626AA	2625BA	2	<i>Erucastrum griquense</i>	x
					<i>Lepidium</i> sp.	x
2625BC	2625BD	2625CB	2625DB	0	None	
2625BD	2625BC	2625DB	2625DA	0	None	
2625CA	2625CC	2625CB	2625AC	1	<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2625CB	2625CD	2625BC	2625DC	0	None	
2625CC	2625CA	2725CB	2625CD	0	<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	
2625CD	2625CB	2625DC	2625DA	0	None	
2625DA	2625BC	2625BD	2625CB	0	None	
2625DB	2625DC	2625BD	2625BC	0	None	
2625DC	2625CD	2625DB	2625CB	0	None	
2625DD	2625DB	2725BB	2725BA	6	<i>Coronopus integrifolius</i>	x
					<i>Erucastrum strigosum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluiatilis</i> var. <i>caledonica</i>	x
					<i>Rorippa nudiuscula</i>	x
2626AA	2625BB	2526CC	2626AB	4	<i>Erucastrum griquense</i>	
					<i>Erucastrum strigosum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium</i> sp.	
					<i>Raphanus raphanistrum</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2626AB	2626AA	2626AC	2626BC	2	<i>Erucastrum griquense</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Lepidium</i> sp.	x
2626AC	2626AB	2626AA	2625BB	2	<i>Erucastum griquense</i>	x
					<i>Lepidium</i> sp.	x
2626AD	2626CA	2626CB	2626BC	0	None	
2626BA	2626BB	2526DC	2526CD	1	<i>Lepidium africanum</i>	x
2626BB	2626BA	2627AA	2526DC	3	<i>Coronopus integrifolius</i>	x
					<i>Erucastum strigosum</i>	x
					<i>Rorippa nudiuscula</i>	x
2626BC	2626BD	2626AD	2626DA	5	<i>Erucastum austroafricanum</i>	x
					<i>Erucastum</i> sp.	x
					<i>Erucastum strigosum</i>	x
					<i>Heliophila suavisissima</i>	x
					<i>Lepidium transvaalense</i>	x
2626BD	2626BC	2627AC	2626DA	2	<i>Erucastum austroafricanum</i>	
					<i>Erucastum</i> sp.	
					<i>Erucastum strigosum</i>	
					<i>Heliophila suavisissima</i>	
					<i>Lepidium transvaalense</i>	
					<i>Lepidium bonariense</i>	x
					<i>Nasturtium officinale</i>	x
2626CA	2626CB	2626CD	2626AC	2	<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus didymus</i>	x
2626CB	2626CD	2626DC	2626CA	5	<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus didymus</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Sisymbrium</i> sp.	x
2626CC	2726AA	2626CD	2725BB	8	<i>Brassica juncea</i>	
					<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus didymus</i>	x
					<i>Coronopus integrifolius</i>	x
					<i>Erucastum strigosum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	x
					<i>Rorippa nudiuscula</i>	x
2626CD	2626CB	2626CC	2726AA	1	<i>Capsella bursa-pastoris</i>	
					<i>Coronopus didymus</i>	
					<i>Brassica juncea</i>	x
2626DA	2626BC	2626BD	2626DC	7	<i>Erucastum austroafricanum</i>	x
					<i>Erucastum</i> sp.	x
					<i>Erucastum strigosum</i>	x
					<i>Heliophila suavisissima</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Sisymbrium</i> sp.	x
2626DB	2627AC	2626BD	2626DA	7	<i>Erucastum austroafricanum</i>	x
					<i>Erucastum</i> sp.	x
					<i>Erucastum strigosum</i>	x
					<i>Heliophila suavisissima</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Nasturtium officinale</i>	x
2626DC	2626CD	2626DB	2626CB	2	<i>Lepidium transvaalense</i>	
					<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus didymus</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	
					<i>Sisymbrium</i> sp.	
2626DD	2626DB	2626DC	2727AA	3	<i>Lepidium transvaalense</i>	x
					<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Sisymbrium</i> sp.	x
2627AA	2626BB	2627AB	2526DD	0	<i>Coronopus integrifolius</i>	
					<i>Erucastum strigosum</i>	
					<i>Rorippa nudiuscula</i>	
2627AB	2627BA	2627AA	2627BC	14	<i>Coronopus integrifolius</i>	x
					<i>Erucastum austroafricanum</i>	x
					<i>Erucastum strigosum</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Heliophila carnosa</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
					<i>Sisymbrium officinale</i>	x
					<i>Sisymbrium orientale</i>	x
					<i>Sisymbrium</i> sp.	x
					<i>Sisymbrium thellungii</i>	x
2627AC	2626BD	2627AD	2626BB	8	<i>Erucastrum austroafricanum</i>	x
					<i>Erucastrum</i> sp.	x
					<i>Erucastrum strigosum</i>	x
					<i>Heliophila suavissima</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	
					<i>Lepidium transvaalense</i>	x
					<i>Nasturtium officinale</i>	
					<i>Raphanus raphanistrum</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2627AD	2627BC	2627CA	2627AC	15	<i>Erucastrum austroafricanum</i>	
					<i>Brassica nigra</i>	x
					<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus integrifolius</i>	x
					<i>Diploaxis muralis</i>	x
					<i>Diploaxis</i> sp.	x
					<i>Heliophila</i> sp.	x
					<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Raphanus raphanistrum</i>	
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Sisymbrium orientale</i>	x
					<i>Sisymbrium</i> sp.	x
					<i>Sisymbrium thellungii</i>	x
					<i>Sisymbrium turczaninowii</i>	
2627BA	2627AB	2627BB	2627BC	8	<i>Erucastrum austroafricanum</i>	
					<i>Cardamine africana</i>	x
					<i>Eruca sativa</i>	x
					<i>Erucastrum strigosum</i>	
					<i>Heliophila carnosa</i>	
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	
					<i>Lepidium ecklonii</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium transvaalense</i>	
					<i>Lepidium virginicum</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Raphanus raphanistrum</i>	
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Rorippa nudiuscula</i>	
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	
					<i>Sisymbrium officinale</i>	
					<i>Sisymbrium orientale</i>	
					<i>Sisymbrium</i> sp.	
					<i>Sisymbrium thellungii</i>	
2627BB	2628AA	2627BA	2527DD	24	<i>Cardamine africana</i>	
					<i>Alyssum minutum</i>	x
					<i>Armoracia rusticana</i>	x
					<i>Brassica rapa</i>	x
					<i>Capsella bursa-pastoris</i>	x
					<i>Cardamine hirsuta</i>	x
					<i>Coronopus didymus</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Coronopus integrifolius</i>	x
					<i>Diplotaxis muralis</i>	x
					<i>Eruca sativa</i>	
					<i>Erucastrum austroafricanum</i>	x
					<i>Erucastrum strigosum</i>	x
					<i>Erysimum cheiri</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila juncea</i>	x
					<i>Heliophila rigidiuscula</i>	
					<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	
					<i>Lepidium ecklonii</i>	
					<i>Lepidium mossii</i>	x
					<i>Lepidium schinzii</i>	
					<i>Lepidium transvaalense</i>	x
					<i>Lepidium virginicum</i>	
					<i>Lobularia maritima</i>	x
					<i>Matthiola incana</i>	x
					<i>Nasturtium officinale</i>	
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	
					<i>Rorippa nudiuscula</i>	
					<i>Sinapis alba</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium officinale</i>	x
					<i>Sisymbrium orientale</i>	
					<i>Sisymbrium</i> sp.	x
					<i>Sisymbrium thellungii</i>	
					<i>Sisymbrium turczaninowii</i>	x
2627BC	2627AD	2627BD	2627DA	10	<i>Erucastrum austroafricanum</i>	
					<i>Erucastrum strigosum</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium mossii</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium orientale</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2627BD	2627BC	2628AC	2627BA	6	<i>Erucastrum austroafricanum</i>	x
					<i>Erucastrum strigosum</i>	
					<i>Heliophila carnosa</i>	
					<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	x
					<i>Lepidium mossii</i>	
					<i>Lepidium transvaalense</i>	
					<i>Nasturtium officinale</i>	
					<i>Raphanus raphanistrum</i>	
					<i>Rorippa nudiuscula</i>	
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
					<i>Sisymbrium officinale</i>	x
					<i>Sisymbrium orientale</i>	
					<i>Sisymbrium</i> sp.	x
					<i>Sisymbrium thellungii</i>	x
2627CA	2627CB	2627CC	2626DD	3	<i>Brassica nigra</i>	
					<i>Capsella bursa-pastoris</i>	
					<i>Coronopus integrifolius</i>	
					<i>Diplotaxis muralis</i>	
					<i>Diplotaxis</i> sp.	
					<i>Erucastrum austroafricanum</i>	
					<i>Heliophila rigidiuscula</i>	x
					<i>Heliophila</i> sp.	
					<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	
					<i>Lepidium schinzii</i>	
					<i>Lepidium</i> sp.	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Lepidium transvaalense</i>	x
					<i>Nasturtium officinale</i>	
					<i>Raphanus raphanistrum</i>	
					<i>Rapistrum rugosum</i>	
					<i>Rorippa fluiatilil var. caledonica</i>	
					<i>Rorippa fluiatilil var. fluiatilil</i>	
					<i>Sisymbrium orientale</i>	
					<i>Sisymbrium sp.</i>	
					<i>Sisymbrium thellungii</i>	
2627CB	2627CA	2627BC	2627CC	16	<i>Brassica nigra</i>	x
					<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus integrifolius</i>	x
					<i>Diplotaxis muralis</i>	x
					<i>Diplotaxis sp.</i>	x
					<i>Erucastrum austroafricanum</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Heliophila sp.</i>	x
					<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium sp.</i>	
					<i>Lepidium transvaalense</i>	
					<i>Nasturtium officinale</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluiatilil var. caledonica</i>	x
					<i>Rorippa fluiatilil var. fluiatilil</i>	
					<i>Sisymbrium orientale</i>	x
					<i>Sisymbrium sp.</i>	x
					<i>Sisymbrium thellungii</i>	x
2627CC	2627CA	2627CB	2627CD	20	<i>Nasturtium officinale</i>	
					<i>Brassica nigra</i>	x
					<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus integrifolius</i>	x
					<i>Diplotaxis muralis</i>	x
					<i>Diplotaxis sp.</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Heliophila sp.</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium sp.</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluiatilil var. caledonica</i>	x
					<i>Rorippa fluiatilil var. fluiatilil</i>	x
					<i>Sisymbrium orientale</i>	x
					<i>Sisymbrium sp.</i>	x
					<i>Sisymbrium thellungii</i>	x
2627CD	2727AB	2727AA	2627CC	1	<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	
					<i>Nasturtium officinale</i>	
					<i>Rorippa fluiatilil var. fluiatilil</i>	
					<i>Sisymbrium sp.</i>	x
2627DA	2627BD	2627DC	2627DB	16	<i>Erucastrum strigosum</i>	x
					<i>Coronopus didymus</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila refracta</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium mossii</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Raphanus raphanistrum</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium orientale</i>	x
2627DB	2627DA	2627DC	2628CA	8	<i>Coronopus didymus</i>	
					<i>Erucastum austroafricanum</i>	
					<i>Heliophila carnosa</i>	x
					<i>Heliophila refracta</i>	
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	
					<i>Rorippa nudiuscula</i>	
					<i>Sisymbrium turczaninowii</i>	x
2627DC	2627DD	2627CD	2727BA	10	<i>Erucastum austroafricanum</i>	
					<i>Coronopus integrifolius</i>	x
					<i>Diplotaxis muralis</i>	x
					<i>Eruca sativa</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	
					<i>Lepidium schinzii</i>	
					<i>Nasturtium officinale</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Rorippa nudiuscula</i>	
					<i>Sisymbrium officinale</i>	x
					<i>Sisymbrium thellungii</i>	x
2627DD	2727BA	2727BB	2627DC	2	<i>Coronopus integrifolius</i>	
					<i>Diplotaxis muralis</i>	
					<i>Eruca sativa</i>	
					<i>Erucastum austroafricanum</i>	
					<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	
					<i>Lepidium schinzii</i>	x
					<i>Nasturtium officinale</i>	
					<i>Raphanus raphanistrum</i>	
					<i>Rapistrum rugosum</i>	
					<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	
					<i>Rorippa nudiuscula</i>	
					<i>Sisymbrium officinale</i>	
					<i>Sisymbrium thellungii</i>	x
2628AA	2627BB	2628AD	2528CC	8	<i>Alyssum minutum</i>	
					<i>Armoracia rusticana</i>	
					<i>Brassica rapa</i>	
					<i>Capsella bursa-pastoris</i>	
					<i>Cardamine africana</i>	x
					<i>Cardamine hirsuta</i>	
					<i>Coronopus didymus</i>	
					<i>Coronopus integrifolius</i>	
					<i>Diplotaxis muralis</i>	
					<i>Eruca sativa</i>	x
					<i>Erucastum austroafricanum</i>	
					<i>Erucastum strigosum</i>	
					<i>Erysimum cheiri</i>	
					<i>Heliophila carnosa</i>	
					<i>Heliophila juncea</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Heliophila</i> sp.	x
					<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	
					<i>Lepidium capense</i>	x
					<i>Lepidium ecklonii</i>	x
					<i>Lepidium mossii</i>	

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Lepidium schinzii</i>	
					<i>Lepidium transvaalense</i>	
					<i>Lepidium virginicum</i>	
					<i>Lobularia maritima</i>	
					<i>Matthiola incana</i>	
					<i>Nasturtium officinale</i>	
					<i>Raphanus raphanistrum</i>	
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sinapis alba</i>	
					<i>Sisymbrium capense</i>	
					<i>Sisymbrium orientale</i>	
					<i>Sisymbrium thellungii</i>	
					<i>Sisymbrium turczaninowii</i>	
2628AB	2628BA	2628AA	2628BC	24	<i>Coronopus integrifolius</i>	
					<i>Alyssum minutum</i>	x
					<i>Armoracia rusticana</i>	x
					<i>Brassica rapa</i>	x
					<i>Capsella bursa-pastoris</i>	x
					<i>Cardamine hirsuta</i>	x
					<i>Coronopus didymus</i>	x
					<i>Descurainia sophia</i>	
					<i>Diplotaxis muralis</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Erucastrum strigosum</i>	x
					<i>Erysimum cheiri</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila juncea</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	
					<i>Lepidium mossii</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium transvaalense</i>	
					<i>Lepidium virginicum</i>	x
					<i>Lobularia maritima</i>	x
					<i>Matthiola incana</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Raphanus raphanistrum</i>	
					<i>Rorippa nudiuscula</i>	
					<i>Sinapis alba</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium orientale</i>	
					<i>Sisymbrium thellungii</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2628AC	2627BD	2628CA	2628AD	12	<i>Coronopus didymus</i>	x
					<i>Erucastrum strigosum</i>	
					<i>Heliophila carnosa</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Heliophila</i> sp.	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium mossii</i>	x
					<i>Lepidium transvaalense</i>	
					<i>Nasturtium officinale</i>	
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium orientale</i>	x
					<i>Sisymbrium thellungii</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2628AD	2628CB	2628CA	2628AC	4	<i>Coronopus didymus</i>	x
					<i>Erucastrum strigosum</i>	x
					<i>Heliophila carnosa</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Heliophila</i> sp.	
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Lepidium transvaalense</i>	
					<i>Nasturtium officinale</i>	
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium thellungii</i>	
					<i>Sisymbrium turczaninowii</i>	
2628BA	2628AB	2628BD	2628BB	8	<i>Coronopus integrifolius</i>	x
					<i>Descurainia sophia</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Heliophila</i> sp.	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium orientale</i>	x
2628BB	2628BA	2529CC	2629AA	3	<i>Coronopus didymus</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
2628BC	2628BD	2628AB	2628DB	8	<i>Coronopus integrifolius</i>	x
					<i>Descurainia sophia</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Heliophila</i> sp.	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium orientale</i>	x
2628BD	2628BC	2629AC	2628DB	0	<i>Heliophila</i> sp.	
					<i>Lepidium bonariense</i>	
					<i>Rorippa nudiuscula</i>	
2628CA	2628CB	2627DB	2628AD	7	<i>Coronopus didymus</i>	
					<i>Erucastrum austroafricanum</i>	x
					<i>Heliophila carnosa</i>	
					<i>Heliophila refracta</i>	x
					<i>Heliophila rigidiuscula</i>	
					<i>Heliophila</i> sp.	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium transvaalense</i>	
					<i>Nasturtium officinale</i>	
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Rorippa nudiuscula</i>	
					<i>Sisymbrium thellungii</i>	x
					<i>Sisymbrium turczaninowii</i>	
2628CB	2628DA	2628CA	2628CC	7	<i>Coronopus didymus</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium africanum</i>	
					<i>Lepidium transvaalense</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Rorippa nudiuscula</i>	
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
					<i>Sisymbrium turczaninowii</i>	
2628CC	2628CB	2628CD	2628CA	7	<i>Coronopus didymus</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Nasturtium officinale</i>	
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	
					<i>Sisymbrium turczaninowii</i>	x
2628CD	2628CC	2628DC	2628DA	3	<i>Erucastrum austroafricanum</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2628DA	2628CB	2628DC	2628DB	3	<i>Erucastrum austroafricanum</i>	
					<i>Lepidium africanum</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2628DB	2629CA	2628DD	2628DC	1	<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
2628DC	2628CD	2628DD	2628DA	1	<i>Erucastum austroafricanum</i>	x
2628DD	2629CC	2729AA	2628DC	4	<i>Diplotaxis muralis</i>	x
					<i>Diplotaxis muralis</i> subsp. <i>Muralis</i>	x
					<i>Sinapis arvensis</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2629AA	2529CC	2629AB	2628BB	2	<i>Coronopus didymus</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	
2629AB	2629AA	2629BA	2529CC	4	<i>Coronopus didymus</i>	x
					<i>Erucastum austroafricanum</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
2629AC	2628BD	2629AD	2629CA	6	<i>Erucastum austroafricanum</i>	x
					<i>Heliophila</i> sp.	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Rorippa nudiuscula</i>	x
2629AD	2629AC	2629BC	2629CA	0	<i>Erucastum austroafricanum</i>	
					<i>Lepidium transvaalense</i>	
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	
2629BA	2629AB	2629BB	2529DD	1	<i>Erucastum austroafricanum</i>	
					<i>Heliophila carnosa</i>	x
2629BB	2629BA	2630AA	2630AC	2	<i>Erucastum austroafricanum</i>	x
					<i>Heliophila carnosa</i>	
					<i>Heliophila rigidiuscula</i>	x
2629BC	2629AD	2629BD	2629BB	4	<i>Erucastum austroafricanum</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
2629BD	2629BC	2629DA	2630CA	3	<i>Cardamine africana</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Rorippa nudiuscula</i>	x
2629CA	2629CD	2629CB	2629BD	9	<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	
					<i>Erucastum austroafricanum</i>	x
					<i>Heliophila</i> sp.	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sinapis arvensis</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2629CB	2629CA	2629CD	2629DA	7	<i>Erucastum austroafricanum</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Sinapis arvensis</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2629CC	2628DD	2629CD	2729AA	8	<i>Diplotaxis muralis</i>	x
					<i>Diplotaxis muralis</i> subsp. <i>Muralis</i>	x
					<i>Erucastum austroafricanum</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Sinapis arvensis</i>	
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2629CD	2629CB	2629CC	2729AB	0	<i>Erucastum austroafricanum</i>	
					<i>Lepidium transvaalense</i>	
					<i>Nasturtium officinale</i>	
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	
					<i>Sinapis arvensis</i>	
					<i>Sisymbrium capense</i>	
					<i>Sisymbrium turczaninowii</i>	
2629DA	2629CB	2629CD	2629DC	7	<i>Erucastum austroafricanum</i>	x
					<i>Lepidium transvaalense</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Nasturtium officinale</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Sinapis arvensis</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2629DB	2629DD	2629DA	2629DC	0	<i>Brassica rapa</i>	
					<i>Erucastrum austroafricanum</i>	
					<i>Lepidium schinzii</i>	
					<i>Lepidium transvaalense</i>	
					<i>Rorippa nudiuscula</i>	
					<i>Sisymbrium turczaninowii</i>	
					<i>Turritis glabra</i>	
2629DC	2629DA	2729BA	2629CD	7	<i>Erucastrum austroafricanum</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Sinapis arvensis</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2629DD	2629DB	2729BB	2729BA	7	<i>Brassica rapa</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium turczaninowii</i>	x
					<i>Turritis glabra</i>	x
2630AA	2629BB	2529DD	2630AC	1	<i>Heliophila rigidiuscula</i>	
					<i>Heliophila carnosa</i>	x
2630AB	2530CC	2630BC	2630AA	1	<i>Lepidium</i> sp.	
					<i>Heliophila rigidiuscula</i>	x
2630AC	2630AA	2630AD	2629BB	2	<i>Heliophila carnosa</i>	x
					<i>Heliophila rigidiuscula</i>	x
2630AD	2630AC	2630AA	2630AB	2	<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium</i> sp.	x
2630BA	2630BB	2630AB	2630BC	3	<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium virginicum</i>	x
					<i>Lepidium</i> sp.	x
2630BB	2630BA	2631AA	2530DC	0	<i>Heliophila rigidiuscula</i>	
					<i>Lepidium virginicum</i>	
2630BC	2630AB	2630DA	2630BA	1	<i>Lepidium</i> sp.	x
2630CA	2630CB	2630AC	2630CD	0	<i>Cardamine africana</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Rorippa nudiuscula</i>	
2630CB	2630CA	2630CD	2630AC	3	<i>Cardamine africana</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Rorippa nudiuscula</i>	x
2630CC	2729BB	2730AA	2629DB	7	<i>Brassica rapa</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Lepidium trifurcum</i>	
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium turczaninowii</i>	x
					<i>Turritis glabra</i>	x
2630CD	2630CB	2730AB	2630CA	4	<i>Cardamine africana</i>	x
					<i>Heliophila linearis</i> var. <i>linearis</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Rorippa nudiuscula</i>	x
2630DA	2630BC	2630DC	2630DD	0	None	
2630DC	2630DA	2630DD	2630CB	0	None	
2630DD	2630DA	2630DC	2630DB	0	None	
2720AA	2620CC	2720AC	2620CD	0	None	
2720AB	2720AD	2720BC	2720BA	0	None	
2720AC	2720AA	2720CA	2720AB	0	None	
2720AD	2720AB	2720BC	2720DA	0	None	
2720BA	2720AB	2720AD	2720BB	0	None	
2720BB	2720BA	2721AA	2621CC	0	None	
2720BC	2720BD	2720DA	2720DB	0	None	

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
2720BD	2720BC	2720DA	2720DB	0	None	
2720CA	2720AC	2720CB	2720CC	0	None	
2720CB	2720CA	2720CC	2720CD	0	None	
2720CC	2720CA	2720CB	2720CD	0	None	
2720CD	2720CA	2720CB	2720CC	0	None	
2720DA	2720BC	2720BD	2720DB	0	None	
2720DB	2720BD	2720DA	2720BC	0	None	
2720DC	2720CD	2720DD	2720CB	0	None	
2720DD	2720DC	2821AA	2721CC	0	None	
2721AA	2721AB	2720BB	2621CC	1	<i>Heliophila affinis</i>	x
2721AB	2721AA	2721BA	2721AD	0	<i>Heliophila affinis</i>	
2721AC	2720BD	2720DB	2720DA	0	None	
2721AD	2721AB	2721BC	2721DA	1	<i>Heliophila affinis</i>	x
2721BA	2721AB	2721AD	2721BC	1	<i>Heliophila affinis</i>	x
2721BB	2721BD	2721BA	2721BD	0	None	
2721BC	2721DA	2721DB	2721AD	0	None	
2721BD	2721BB	2721DB	2722AA	0	None	
2721CA	2721AC	2720BD	2720DB	0	None	
2721CB	2721CA	2721DC	2721BD	0	None	
2721CC	2720DD	2720DB	2821AB	0	None	
2721CD	2721CA	2721CB	2721DC	0	None	
2721DA	2721BC	2721DB	2721AD	0	None	
2721DB	2721DA	2721BC	2721BD	0	None	
2721DC	2721CD	2721CB	2721DD	0	None	
2721DD	2821BB	2821BA	2721DB	0	None	
2722AA	2722AD	2722AC	2722AB	1	<i>Coronopus integrifolius</i>	x
2722AB	2722AD	2622CD	2722BA	1	<i>Coronopus integrifolius</i>	x
2722AC	2722AA	2722CA	2722AD	1	<i>Coronopus integrifolius</i>	x
2722AD	2722AB	2722CB	2722AA	4	<i>Coronopus integrifolius</i>	
					<i>Heliophila suavisissima</i>	x
					<i>Heliophila trifurca</i>	x
					<i>Heliophila variabilis</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2722BA	2722AB	2722BC	2722BD	0	None	
2722BB	2722BA	2722BD	2622DC	0	None	
2722BC	2722BA	2722DA	2722BD	0	None	
2722BD	2722BA	2722BB	2722BC	0	None	
2722CA	2722AC	2722CC	2722CB	4	<i>Heliophila suavisissima</i>	x
					<i>Heliophila trifurca</i>	x
					<i>Heliophila variabilis</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2722CB	2722AD	2722CD	2722CA	1	<i>Coronopus integrifolius</i>	x
					<i>Heliophila suavisissima</i>	
					<i>Heliophila trifurca</i>	
					<i>Heliophila variabilis</i>	
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	
2722CC	2722CA	2722CB	2822AB	4	<i>Heliophila suavisissima</i>	x
					<i>Heliophila trifurca</i>	x
					<i>Heliophila variabilis</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2722CD	2722CB	2822AB	2822CA	4	<i>Heliophila suavisissima</i>	x
					<i>Heliophila trifurca</i>	x
					<i>Heliophila variabilis</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2722DA	2722BC	2722DB	2722DD	0	None	
2722DB	2722BD	2722DA	2722BC	0	None	
2722DC	2722CD	2822BA	2722DD	0	<i>Heliophila carnosa</i>	
2722DD	2822BB	2722DB	2822BA	0	None	
2723AA	2723AD	2723AC	2723AB	5	<i>Coronopus integrifolius</i>	x
					<i>Erucastrum griquense</i>	
					<i>Erucastrum strigosum</i>	x
					<i>Matthiola</i> sp.	x
					<i>Matthiola torulosa</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2723AB	2723AD	2723AA	2723BA	8	<i>Coronopus integrifolius</i>	x
					<i>Erucastrum griquense</i>	x
					<i>Erucastrum strigosum</i>	x
					<i>Heliophila amplexicaulis</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Heliophila suavisissima</i>	x
					<i>Matthiola</i> sp.	x
					<i>Matthiola torulosa</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2723AC	2723AA	2723CA	2723AD	6	<i>Coronopus integrifolius</i>	x
					<i>Erucastrum griquense</i>	x
					<i>Erucastrum strigosum</i>	x
					<i>Matthiola</i> sp.	x
					<i>Matthiola torulosa</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2723AD	2723AA	2723CB	2723AB	1	<i>Coronopus integrifolius</i>	
					<i>Erucastrum griquense</i>	
					<i>Erucastrum strigosum</i>	
					<i>Heliophila minima</i>	x
					<i>Matthiola</i> sp.	
					<i>Matthiola torulosa</i>	
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	
2723BA	2723AD	2723BC	2723AB	6	<i>Coronopus integrifolius</i>	x
					<i>Erucastrum griquense</i>	x
					<i>Erucastrum strigosum</i>	x
					<i>Heliophila amplexicaulis</i>	
					<i>Heliophila suavisissima</i>	
					<i>Matthiola</i> sp.	x
					<i>Matthiola torulosa</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2723BB	2723BD	2723BC	2724AA	5	<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus integrifolius</i>	x
					<i>Erucastrum strigosum</i>	x
					<i>Rorippa fluiatililis</i> var. <i>caledonica</i>	x
					<i>Sisymbrium capense</i>	
					<i>Sisymbrium turczaninowii</i>	x
2723BC	2723BA	2723DA	2723BD	2	<i>Heliophila amplexicaulis</i>	x
					<i>Heliophila suavisissima</i>	x
2723BD	2723BC	2723BB	2723DB	1	<i>Sisymbrium capense</i>	x
2723CA	2723AC	2723CC	2723CB	2	<i>Coronopus integrifolius</i>	x
					<i>Heliophila minima</i>	x
2723CB	2723AD	2723CC	2723CD	6	<i>Coronopus integrifolius</i>	x
					<i>Erucastrum griquense</i>	x
					<i>Erucastrum strigosum</i>	x
					<i>Heliophila minima</i>	
					<i>Matthiola</i> sp.	x
					<i>Matthiola torulosa</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2723CC	2723CB	2723CA	2723CD	1	<i>Coronopus integrifolius</i>	
					<i>Heliophila minima</i>	x
2723CD	2723CB	2823AB	2723CC	2	<i>Coronopus integrifolius</i>	x
					<i>Heliophila minima</i>	x
2723DA	2723DC	2723CB	2723BD	2	<i>Erucastrum griquense</i>	x
					<i>Heliophila minima</i>	x
2723DB	2723BD	2724AC	2723AC	1	<i>Coronopus integrifolius</i>	x
2723DC	2723DA	2823BA	2723DD	5	<i>Erucastrum griquense</i>	
					<i>Brassica</i> sp.	x
					<i>Heliophila suavisissima</i>	x
					<i>Matthiola</i> sp.	x
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2723DD	2724CA	2724DB	2724CC	5	<i>Brassica</i> sp.	
					<i>Coronopus integrifolius</i>	x
					<i>Erucastrum griquense</i>	
					<i>Matthiola</i> sp.	x
					<i>Matthiola torulosa</i>	x
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
					<i>Sisymbrium</i> sp.	x
2724AA	2723BB	2624CC	2724AB	1	<i>Capsella bursa-pastoris</i>	
					<i>Coronopus integrifolius</i>	
					<i>Erucastrum strigosum</i>	
					<i>Rorippa fluiatililis</i> var. <i>caledonica</i>	

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium turczaninowii</i>	
2724AB	2724BA	2724AA	2724AD	3	<i>Capsella bursa-pastoris</i>	
					<i>Coronopus integrifolius</i>	
					<i>Erucastrium strigosum</i>	x
					<i>Rorippa fluiatilis</i> var. <i>caledonica</i>	x
					<i>Sisymbrium capense</i>	
					<i>Sisymbrium turczaninowii</i>	x
2724AC	2724CA	2723DB	2723BD	4	<i>Coronopus integrifolius</i>	
					<i>Matthiola</i> sp.	x
					<i>Matthiola torulosa</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
					<i>Sisymbrium</i> sp.	x
2724AD	2724CA	2724BC	2724CB	4	<i>Matthiola</i> sp.	x
					<i>Matthiola torulosa</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
					<i>Sisymbrium</i> sp.	x
2724BA	2724AB	2724AD	2724BB	3	<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus integrifolius</i>	x
					<i>Sisymbrium capense</i>	x
2724BB	2724BA	2724BC	2624DC	3	<i>Coronopus integrifolius</i>	x
					<i>Heliophila</i> sp.	x
					<i>Sisymbrium turczaninowii</i>	x
2724BC	2724BD	2724DA	2724AD	0	None	
2724BD	2724BC	2724DA	2724BB	0	None	
2724CA	2723DD	2724CC	2724CB	4	<i>Matthiola</i> sp.	
					<i>Brassica</i> sp.	x
					<i>Coronopus integrifolius</i>	x
					<i>Erucastrium griquense</i>	x
					<i>Matthiola torulosa</i>	
					<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	
					<i>Sisymbrium</i> sp.	
2724CB	2724AD	2724CC	2724CD	6	<i>Coronopus integrifolius</i>	x
					<i>Erucastrium griquense</i>	x
					<i>Erucastrium strigosum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Matthiola torulosa</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2724CC	2824AA	2724CA	2723DD	8	<i>Coronopus integrifolius</i>	
					<i>Brassica</i> sp.	x
					<i>Erucastrium griquense</i>	x
					<i>Erucastrium strigosum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Matthiola torulosa</i>	x
					<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
					<i>Sisymbrium</i> sp.	x
2724CD	2724CB	2724CC	2824AA	2	<i>Coronopus integrifolius</i>	
					<i>Brassica</i> sp.	x
					<i>Erucastrium griquense</i>	
					<i>Erucastrium strigosum</i>	
					<i>Heliophila</i> sp.	x
					<i>Lepidium africanum</i>	
					<i>Matthiola torulosa</i>	
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	
2724DA	2724BC	2724CD	2724DC	6	<i>Coronopus integrifolius</i>	x
					<i>Erucastrium griquense</i>	x
					<i>Erucastrium strigosum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Matthiola torulosa</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2724DB	2725CC	2724DD	2724DC	6	<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus integrifolius</i>	x
					<i>Erucastrium austroafricanum</i>	x
					<i>Erucastrium griquense</i>	x
					<i>Lepidium africanum</i>	x
					<i>Matthiola</i> sp.	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Rapistrum rugosum</i>	
2724DC	2724DA	2824BA	2824AB	3	<i>Coronopus integrifolius</i>	x
					<i>Heliophila lactea</i>	x
					<i>Heliophila suavisissima</i>	x
2724DD	2724DB	2725CC	2824BB	10	<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus integrifolius</i>	x
					<i>Erucastrium austroafricanum</i>	x
					<i>Erucastrium griquense</i>	x
					<i>Heliophila remotiflora</i>	x
					<i>Lepidium africanum</i>	x
					<i>Matthiola</i> sp.	x
					<i>Nasturtium officinale</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluiatililis</i> var. <i>caledonica</i>	x
2725AA	2725AC	2625CC	2725AB	1	<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2725AB	2625CC	2725AA	2725AD	1	<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2725AC	2725AA	2725AB	2725CA	0	None	
2725AD	2725CA	2725AC	2725CB	3	<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus integrifolius</i>	x
					<i>Lepidium bonariense</i>	x
2725BA	2725BC	2625DD	2625CD	0	None	
2725BB	2625DD	2626CC	2726AA	1	<i>Coronopus integrifolius</i>	
					<i>Brassica juncea</i>	x
					<i>Erucastrium strigosum</i>	
					<i>Lepidium africanum</i>	
					<i>Rapistrum rugosum</i>	
					<i>Rorippa fluiatililis</i> var. <i>caledonica</i>	
					<i>Rorippa nudiuscula</i>	
2725BC	2725AD	2725BD	2725CB	4	<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus integrifolius</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Rorippa nudiuscula</i>	x
2725BD	2725BC	2725DB	2725BB	5	<i>Rorippa nudiuscula</i>	
					<i>Coronopus integrifolius</i>	x
					<i>Erucastrium strigosum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluiatililis</i> var. <i>caledonica</i>	x
2725CA	2725AC	2725AD	2724DB	1	<i>Rapistrum rugosum</i>	x
2725CB	2725CC	2725CD	2725DA	4	<i>Capsella bursa-pastoris</i>	
					<i>Coronopus integrifolius</i>	
					<i>Erucastrium austroafricanum</i>	x
					<i>Erucastrium griquense</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	
					<i>Matthiola</i> sp.	x
2725CC	2824BB	2725CB	2725CD	4	<i>Capsella bursa-pastoris</i>	
					<i>Coronopus integrifolius</i>	
					<i>Erucastrium austroafricanum</i>	
					<i>Erucastrium griquense</i>	
					<i>Heliophila remotiflora</i>	x
					<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	x
					<i>Matthiola</i> sp.	
					<i>Nasturtium officinale</i>	x
					<i>Rorippa fluiatililis</i> var. <i>caledonica</i>	x
2725CD	2725CC	2725CB	2725DC	7	<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus integrifolius</i>	x
					<i>Erucastrium austroafricanum</i>	x
					<i>Erucastrium griquense</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Matthiola</i> sp.	x
2725DA	2725CB	2725DD	2725DB	3	<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus integrifolius</i>	x
					<i>Lepidium bonariense</i>	x
2725DB	2726CC	2726CA	2725DA	1	<i>Coronopus integrifolius</i>	
					<i>Erucastrium strigosum</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
2725DC	2725CD	2725DB	2725DD	1	<i>Coronopus integrifolius</i>	x
2725DD	2725DA	2726CC	2725DC	1	<i>Erucastrum strigosum</i>	x
2726AA	2626CC	2626CD	2726AB	3	<i>Brassica juncea</i>	x
					<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus didymus</i>	x
2726AB	2626CD	2726AA	2726BA	2	<i>Capsella bursa-pastoris</i>	x
					<i>Coronopus didymus</i>	x
2726AC	2726AD	2725DB	2726CA	2	<i>Coronopus integrifolius</i>	x
					<i>Rorippa fluiatililis</i> var. <i>caledonica</i>	x
2726AD	2726AC	2726BC	2726DA	0	<i>Rorippa fluiatililis</i> var. <i>caledonica</i>	
2726BA	2726AB	2726BB	2626DC	3	<i>Lepidium transvaalense</i>	x
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	x
					<i>Sisymbrium</i> sp.	x
2726BB	2726BA	2727AA	2726BD	0	None	
2726BC	2726AD	2726DA	2726CB	1	<i>Rorippa fluiatililis</i> var. <i>caledonica</i>	x
2726BD	2727AC	2727CA	2726BA	12	<i>Brassica rapa</i>	x
					<i>Brassica</i> sp.	x
					<i>Capsella bursa-pastoris</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sinapis arvensis</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium capense</i> x <i>S. turczaninowii</i>	x
					<i>Sisymbrium orientale</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2726CA	2726CC	2725DB	2725DD	2	<i>Coronopus integrifolius</i>	x
					<i>Erucastrum strigosum</i>	x
2726CB	2726DA	2726AC	2726AD	1	<i>Rorippa fluiatililis</i> var. <i>caledonica</i>	x
2726CC	2725DD	2726CA	2725DB	1	<i>Erucastrum strigosum</i>	
					<i>Coronopus integrifolius</i>	x
2726CD	2726DC	2726CB	2826AA	2	<i>Lepidium</i> sp.	x
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	x
2726DA	2726BC	2726CB	2726DD	1	<i>Lepidium africanum</i>	x
2726DB	2726DA	2726DD	2727CC	1	<i>Lepidium africanum</i>	x
2726DC	2726CD	2726DA	2826BB	0	<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	
2726DD	2726DB	2726DA	2827AA	0	<i>Lepidium africanum</i>	
2727AA	2627CC	2727AB	2727AD	3	<i>Nasturtium officinale</i>	x
					<i>Rorippa fluiatililis</i> var. <i>caledonica</i>	x
					<i>Sisymbrium</i> sp.	x
2727AB	2727BA	2627CD	2727AA	5	<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	x
					<i>Sisymbrium</i> sp.	
					<i>Sisymbrium thellungii</i>	x
2727AC	2727AD	2726BD	2727CA	13	<i>Brassica rapa</i>	x
					<i>Brassica</i> sp.	x
					<i>Capsella bursa-pastoris</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Rorippa fluiatililis</i> var. <i>caledonica</i>	x
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sinapis arvensis</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium capense</i> x <i>S. turczaninowii</i>	x
					<i>Sisymbrium orientale</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2727AD	2727AC	2727CA	2727CB	12	<i>Brassica rapa</i>	x
					<i>Brassica</i> sp.	x
					<i>Capsella bursa-pastoris</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Rorippa fluiatililis</i> var. <i>caledonica</i>	
					<i>Rorippa fluiatililis</i> var. <i>fluiatililis</i>	x
					<i>Rorippa nudiuscula</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Sinapis arvensis</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium capense</i> x <i>S. turczaninowii</i>	x
					<i>Sisymbrium orientale</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2727BA	2727AB	2727BC	2727BD	2	<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	x
					<i>Sisymbrium</i> sp.	x
					<i>Sisymbrium thellungii</i>	
2727BB	2727BD	2727BA	2627DD	14	<i>Coronopus integrifolius</i>	x
					<i>Diplotaxis muralis</i>	x
					<i>Eruca sativa</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	x
					<i>Rorippa fluiatilis</i> var. <i>caledonica</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium officinale</i>	x
					<i>Sisymbrium thellungii</i>	x
2727BC	2727CB	2727DA	2727AD	2	<i>Lepidium africanum</i>	x
					<i>Rorippa fluiatilis</i> var. <i>caledonica</i>	x
2727BD	2727BB	2727DB	2727BA	2	<i>Capsella bursa-pastoris</i>	x
					<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	
					<i>Sisymbrium thellungii</i>	x
2727CA	2727CB	2726DB	2726BD	0	<i>Brassica rapa</i>	
					<i>Brassica</i> sp.	
					<i>Capsella bursa-pastoris</i>	
					<i>Erucastrum austroafricanum</i>	
					<i>Lepidium africanum</i>	
					<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	
					<i>Rorippa nudiuscula</i>	
					<i>Sinapis arvensis</i>	
					<i>Sisymbrium capense</i>	
					<i>Sisymbrium capense</i> x <i>S. turczaninowii</i>	
					<i>Sisymbrium orientale</i>	
					<i>Sisymbrium turczaninowii</i>	
2727CB	2727AC	2727AD	2727DA	1	<i>Lepidium africanum</i>	
					<i>Rorippa fluiatilis</i> var. <i>caledonica</i>	x
2727CC	2727CA	2727CB	2826BB	12	<i>Brassica rapa</i>	x
					<i>Brassica</i> sp.	x
					<i>Capsella bursa-pastoris</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sinapis arvensis</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium capense</i> x <i>S. turczaninowii</i>	x
					<i>Sisymbrium orientale</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2727CD	2727CB	2727DC	2827BA	1	<i>Lepidium africanum</i>	x
2727DA	2727CB	2727BC	2727DC	1	<i>Lepidium africanum</i>	x
2727DB	2727BD	2727DD	2727DA	2	<i>Capsella bursa-pastoris</i>	
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	x
2727DC	2727CD	2727DA	2827AB	0	None	
2727DD	2728CC	2727DB	2727DA	1	<i>Capsella bursa-pastoris</i>	x
					<i>Raphanus raphanistrum</i>	
					<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	
2728AA	2728AC	2728AB	2728AD	0	None	
2728AB	2728AA	2728BC	2728AD	1	<i>Erucastrum austroafricanum</i>	x
2728AC	2728AA	2728AB	2728AD	0	None	
2728AD	2728BC	2728AB	2728AC	1	<i>Erucastrum austroafricanum</i>	x
2728BA	2728BB	2728AB	2728BC	1	<i>Erucastrum austroafricanum</i>	x
2728BB	2728BA	2728BC	2728AA	2	<i>Diplotaxis muralis</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Erucastum austroafricanum</i>	
					<i>Sisymbrium turczaninowii</i>	x
2728BC	2728AD	2728BD	2728DA	2	<i>Erucastum austroafricanum</i>	
					<i>Lepidium africanum</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2728BD	2729AC	2728BC	2728BB	0	<i>Erucastum austroafricanum</i>	
					<i>Lepidium africanum</i>	
					<i>Sisymbrium turczaninowii</i>	
2728CA	2728CC	2728CB	2728CD	3	<i>Lepidium africanum</i>	
					<i>Lepidium transvaalense</i>	
					<i>Lepidium trifurcum</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Rorippa fluiatilis</i> var. <i>caledonica</i>	
					<i>Sisymbrium turczaninowii</i>	x
2728CB	2728DA	2728AC	2728BC	2	<i>Erucastum austroafricanum</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2728CC	2728CA	2727DD	2728CD	8	<i>Lepidium africanum</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Lepidium trifurcum</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa fluiatilis</i> var. <i>caledonica</i>	x
					<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2728CD	2728DC	2828AA	2728CC	1	<i>Heliophila carnos</i>	x
					<i>Lepidium trifurcum</i>	
					<i>Nasturtium officinale</i>	
					<i>Sisymbrium turczaninowii</i>	
2728DA	2728BC	2728CB	2728BD	2	<i>Erucastum austroafricanum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Sisymbrium turczaninowii</i>	
2728DB	2728DA	2728DD	2728BD	5	<i>Erucastum austroafricanum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
					<i>Sisymbrium thellungii</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2728DC	2728CD	2728DD	2828BA	5	<i>Lepidium trifurcum</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
					<i>Sisymbrium thellungii</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2728DD	2728DC	2828BA	2728DB	0	<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	
					<i>Sisymbrium thellungii</i>	
2729AA	2728BB	2629CC	2729AB	2	<i>Diplotaxis muralis</i>	
					<i>Erucastum austroafricanum</i>	x
					<i>Sinapis arvensis</i>	x
					<i>Sisymbrium turczaninowii</i>	
2729AB	2629CC	2629CD	2729AA	9	<i>Diplotaxis muralis</i>	x
					<i>Erucastum austroafricanum</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	x
					<i>Sinapis arvensis</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2729AC	2728BD	2728BB	2728DB	1	<i>Erucastum austroafricanum</i>	
					<i>Lepidium africanum</i>	
					<i>Sisymbrium turczaninowii</i>	x
2729AD	2728AC	2729AA	2728CB	4	<i>Sisymbrium thellungii</i>	
					<i>Diplotaxis muralis</i>	x
					<i>Erucastum austroafricanum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2729BA	2629CD	2629DC	2729AB	7	<i>Erucastum austroafricanum</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Rorippa fluiatilis</i> var. <i>fluiatilis</i>	x
					<i>Sinapis arvensis</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2729BB	2629DD	2729BC	2729BD	3	<i>Erucastrum austroafricanum</i>	
					<i>Brassica rapa</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Rorippa nudiuscula</i>	
					<i>Sisymbrium thellungii</i>	x
					<i>Sisymbrium turczaninowii</i>	
2729BC	2729BB	2729BD	2729DA	6	<i>Brassica rapa</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium thellungii</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2729BD	2729BC	2729BB	2729DA	1	<i>Brassica rapa</i>	
					<i>Erucastrum austroafricanum</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium thellungii</i>	
					<i>Sisymbrium turczaninowii</i>	
2729CA	2728DB	2729CC	2729CD	0	<i>Lepidium transvaalense</i>	
					<i>Raphanus raphanistrum</i>	
2729CB	2729BC	2729DA	2729CA	2	<i>Lepidium transvaalense</i>	x
					<i>Raphanus raphanistrum</i>	x
2729CC	2729CA	2729CD	2729BB	2	<i>Lepidium transvaalense</i>	x
					<i>Raphanus raphanistrum</i>	x
2729CD	2729CC	2729CA	2829AB	2	<i>Lepidium transvaalense</i>	x
					<i>Raphanus raphanistrum</i>	x
2729DA	2729CB	2729BC	2729BD	5	<i>Brassica rapa</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Sisymbrium thellungii</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2729DC	2729CD	2829AB	2829BA	0	<i>Heliophila cornuta</i>	
					<i>Heliophila rigidiuscula</i>	
2730AA	2630CC	2730AC	2730AD	8	<i>Capsella bursa-pastoris</i>	x
					<i>Cardamine impatiens</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium trifurcum</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium sp.</i>	x
2730AB	2730BA	2730AD	2630CD	4	<i>Cardamine impatiens</i>	x
					<i>Heliophila linearis</i> var. <i>linearis</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium sp.</i>	x
2730AC	2730AA	2730AD	2730CB	4	<i>Capsella bursa-pastoris</i>	
					<i>Cardamine impatiens</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Lepidium schinzii</i>	
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium sp.</i>	
2730AD				–	<i>Cardamine impatiens</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Raphanus raphanistrum</i>	
					<i>Rorippa nudiuscula</i>	
					<i>Sisymbrium sp.</i>	
2730BA	2730AB	2630DC	2730BB	2	<i>Heliophila linearis</i> var. <i>linearis</i>	x
					<i>Heliophila rigidiuscula</i>	x
2730BB	2730BA	2731AA	2630DD	0	None	
2820AA	2720CC	2820AB	2820AD	3	<i>Heliophila deserticola</i>	x
					<i>Heliophila trifurca</i>	x
					<i>Heliophila variabilis</i>	x
2820AB	2820AA	2820BA	2820BC	0	None	

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
2820AC	2820AD	2820AA	2820CB	9	<i>Coronopus didymus</i>	x
					<i>Coronopus integrifolius</i>	x
					<i>Heliophila deserticola</i>	x
					<i>Heliophila minima</i>	x
					<i>Heliophila seselifolia</i> var. <i>seselifolia</i>	x
					<i>Heliophila trifurca</i>	
					<i>Heliophila variabilis</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium desertorum</i>	x
					<i>Sisymbrium capense</i>	x
2820AD	2820AC	2820BC	2820AA	0	<i>Heliophila deserticola</i>	
					<i>Heliophila trifurca</i>	
					<i>Heliophila variabilis</i>	
2820BA	2820AB	2820BB	2820BD	0	None	
2820BB	2820BA	2820BC	2820BD	0	None	
2820BC	2820AD	2820AB	2820BB	3	<i>Heliophila deserticola</i>	x
					<i>Heliophila trifurca</i>	x
					<i>Heliophila variabilis</i>	x
2820BD	2820BA	2820BC	2820BB	0	None	
2820CA	2819BD	2820CB	2820AC	7	<i>Heliophila deserticola</i>	
					<i>Coronopus didymus</i>	x
					<i>Coronopus integrifolius</i>	x
					<i>Heliophila minima</i>	x
					<i>Heliophila seselifolia</i> var. <i>seselifolia</i>	x
					<i>Heliophila trifurca</i>	
					<i>Lepidium africanum</i>	x
					<i>Lepidium desertorum</i>	x
					<i>Sisymbrium capense</i>	x
2820CB	2820AC	2820CA	2820DC	1	<i>Coronopus didymus</i>	
					<i>Coronopus integrifolius</i>	
					<i>Heliophila deserticola</i>	
					<i>Heliophila minima</i>	
					<i>Heliophila seselifolia</i> var. <i>seselifolia</i>	
					<i>Heliophila trifurca</i>	
					<i>Heliophila variabilis</i>	x
					<i>Lepidium africanum</i>	
					<i>Lepidium desertorum</i>	
					<i>Sisymbrium capense</i>	
2820CC	2820CD	2920AA	2920AB	0	<i>Heliophila deserticola</i>	
2820CD	2820CC	2920AA	2920AB	1	<i>Heliophila deserticola</i>	x
2820DA	2820DB	2820CB	2820DD	9	<i>Coronopus didymus</i>	x
					<i>Coronopus integrifolius</i>	x
					<i>Heliophila deserticola</i>	x
					<i>Heliophila minima</i>	x
					<i>Heliophila seselifolia</i> var. <i>seselifolia</i>	x
					<i>Heliophila trifurca</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium desertorum</i>	x
					<i>Sisymbrium capense</i>	x
2820DB	2821AC	2820DA	2820DD	0	None	
2820DC	2820CB	2820DD	2820DB	8	<i>Coronopus didymus</i>	x
					<i>Coronopus integrifolius</i>	x
					<i>Heliophila deserticola</i>	
					<i>Heliophila minima</i>	x
					<i>Heliophila seselifolia</i> var. <i>seselifolia</i>	x
					<i>Heliophila trifurca</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium desertorum</i>	x
					<i>Sisymbrium capense</i>	x
2820DD	2820DA	2820DB	2820DC	1	<i>Heliophila deserticola</i>	x
2821AA	2720DD	2820BD	2821AC	0	None	
2821AB	2821BA	2721CC	2821AC	0	None	
2821AC	2821AA	2821AD	2821AB	1	<i>Heliophila minima</i>	x
2821AD	2821AC	2821BC	2821AA	1	<i>Coronopus integrifolius</i>	x
					<i>Heliophila minima</i>	
2821BA	2821AB	2821BB	2821BD	0	None	
2821BB	2821BA	2721DD	2821BD	0	None	
2821BC	2821AD	2821DB	2821DA	1	<i>Coronopus integrifolius</i>	

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Heliophila minima</i>	x
2821BD	2821BB	2821DB	2821BA	0	None	
2821CA	2820DB	2821CB	2821CC	0	None	
2821CB	2821CA	2821CC	2821CD	0	None	
2821CC	2821CD	2921AA	2920BB	0	None	
2821CD	2921BA	2921AA	2821DC	0	None	
2821DA	2821DC	2821DD	2821BC	1	<i>Coronopus integrifolius</i>	x
2821DB	2821BC	2821DD	2821DA	1	<i>Coronopus integrifolius</i>	x
2821DC	2821DA	2821DD	2921BA	0	None	
2821DD	2922AA	2821DA	2921BB	0	None	
2822AA	2722CC	2822AC	2821BB	0	None	
2822AB	2722CD	2822BA	2822AD	0	None	
2822AC	2822CA	2821BB	2822AB	0	None	
2822AD	2822AB	2822CB	2822BC	3	<i>Heliophila trifurca</i>	x
					<i>Lepidium africanum</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2822BA	2722CD	2822BC	2822AB	2	<i>Heliophila trifurca</i>	x
					<i>Lepidium africanum</i>	x
2822BB	2722DD	2822BD	2823AC	8	<i>Coronopus integrifolius</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila suavisissima</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium desertorum</i>	x
					<i>Lepidium ruderales</i>	x
					<i>Matthiola torulosa</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2822BC	2822BA	2822DA	2822CB	2	<i>Coronopus integrifolius</i>	x
					<i>Heliophila trifurca</i>	
					<i>Lepidium africanum</i>	
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2822BD	2823AC	2822BB	2822DB	3	<i>Coronopus integrifolius</i>	
					<i>Heliophila carnosa</i>	x
					<i>Heliophila suavisissima</i>	x
					<i>Lepidium africanum</i>	
					<i>Lepidium desertorum</i>	
					<i>Lepidium ruderales</i>	
					<i>Matthiola torulosa</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	
2822CA	2822AC	2822CB	2822CD	1	<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2822CB	2822AD	2822BC	2822CD	2	<i>Heliophila trifurca</i>	x
					<i>Lepidium africanum</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	
2822CC	2822CD	2822CA	2821DB	0	None	
2822CD	2822CA	2822DA	2822CB	2	<i>Coronopus integrifolius</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2822DA	2822BC	2822CB	2822CD	3	<i>Coronopus integrifolius</i>	
					<i>Heliophila trifurca</i>	x
					<i>Lepidium africanum</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2822DB	2823CA	2822DD	2823CC	2	<i>Lepidium desertorum</i>	
					<i>Lepidium pinnatum</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2822DC	2822DD	2822DA	2922BB	1	<i>Coronopus integrifolius</i>	x
2822DD	2822DC	2823CC	2823CA	2	<i>Lepidium pinnatum</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2823AA	2723CC	2723CD	2823AB	1	<i>Lepidium africanum</i>	
					<i>Lepidium trifurcum</i>	
					<i>Coronopus integrifolius</i>	x
2823AB	2723CD	2723DC	2723CC	2	<i>Coronopus integrifolius</i>	x
					<i>Erucastrum griquense</i>	x
2823AC	2823AA	2822BD	2823AD	8	<i>Heliophila carnosa</i>	
					<i>Coronopus integrifolius</i>	x
					<i>Erucastrum austroafricanum</i>	x
					<i>Erucastrum strigosum</i>	x
					<i>Heliophila suavisissima</i>	
					<i>Lepidium africanum</i>	x
					<i>Lepidium desertorum</i>	x
					<i>Lepidium ruderales</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Lepidium trifurcum</i>	x
					<i>Matthiola torulosa</i>	
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2823AD	2823AC	2823CA	2823CB	3	<i>Erucastrum austroafricanum</i>	
					<i>Erucastrum strigosum</i>	
					<i>Heliophila carnosa</i>	x
					<i>Heliophila suavisissima</i>	x
					<i>Matthiola torulosa</i>	x
2823BA	2723DC	2823BB	2823AB	1	<i>Heliophila suavisissima</i>	
					<i>Erucastrum griquense</i>	x
					<i>Matthiola</i> sp.	
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	
2823BB	2824AA	2724CC	2823BD	4	<i>Brassica</i> sp.	x
					<i>Coronopus integrifolius</i>	x
					<i>Erucastrum strigosum</i>	x
					<i>Heliophila</i> sp.	x
2823BC	2823DA	2823BD	2823BB	0	None	
2823BD	2823BB	2823BC	2823DA	0	None	
2823CA	2823CC	2822DD	2823AD	2	<i>Erucastrum austroafricanum</i>	x
					<i>Erucastrum strigosum</i>	x
2823CB	2823AD	2823CA	2823CC	4	<i>Erucastrum austroafricanum</i>	x
					<i>Erucastrum strigosum</i>	x
					<i>Lepidium pinnatum</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2823CC	2822DD	2823CA	2923AA	0	<i>Lepidium pinnatum</i>	
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	
2823CD	2823CB	2823DA	2823DC	3	<i>Coronopus integrifolius</i>	x
					<i>Erucastrum griquense</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2823DA	2823BC	2823BD	2823CD	0	None	
2823DB	2823DC	2824AC	2823BD	4	<i>Coronopus integrifolius</i>	x
					<i>Erucastrum griquense</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2823DC	2823DB	2823DD	2823DA	1	<i>Coronopus integrifolius</i>	
					<i>Erucastrum griquense</i>	
					<i>Rapistrum rugosum</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	
2823DD	2824CA	2823DC	2823DB	3	<i>Coronopus integrifolius</i>	x
					<i>Erucastrum griquense</i>	x
					<i>Rapistrum rugosum</i>	
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2824AA	2724CD	2823BB	2724CC	5	<i>Brassica</i> sp.	
					<i>Coronopus integrifolius</i>	x
					<i>Erucastrum griquense</i>	x
					<i>Erucastrum strigosum</i>	
					<i>Heliophila</i> sp.	
					<i>Lepidium africanum</i>	x
					<i>Matthiola torulosa</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2824AB	2824AC	2724DC	2724CD	7	<i>Coronopus integrifolius</i>	x
					<i>Erucastrum griquense</i>	x
					<i>Erucastrum strigosum</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Matthiola torulosa</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2824AC	2824AB	2823DB	2823BB	0	<i>Erucastrum griquense</i>	
					<i>Lepidium schinzii</i>	
2824AD	2824CA	2824BA	2824AB	2	<i>Coronopus integrifolius</i>	
					<i>Erucastrum griquense</i>	
					<i>Heliophila lactea</i>	x
					<i>Heliophila suavisissima</i>	x
					<i>Lepidium desertorum</i>	
					<i>Lepidium myriocarpum</i>	
2824BA	2824AD	2824BD	2824BB	7	<i>Coronopus integrifolius</i>	
					<i>Capsella bursa-pastoris</i>	x
					<i>Erucastrum griquense</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Heliophila lactea</i>	
					<i>Heliophila remotiflora</i>	x
					<i>Heliophila suavisissima</i>	
					<i>Lepidium desertorum</i>	x
					<i>Lepidium myriocarpum</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	x
2824BB	2824BC	2824BD	2824BA	3	<i>Capsella bursa-pastoris</i>	
					<i>Coronopus integrifolius</i>	
					<i>Erucastrum griquense</i>	
					<i>Heliophila lactea</i>	x
					<i>Heliophila remotiflora</i>	
					<i>Heliophila suavisissima</i>	x
					<i>Nasturtium officinale</i>	
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	
2824BC	2824BB	2824BD	2824DA	4	<i>Coronopus integrifolius</i>	
					<i>Capsella bursa-pastoris</i>	x
					<i>Erucastrum griquense</i>	
					<i>Heliophila remotiflora</i>	x
					<i>Lepidium</i> sp.	x
					<i>Nasturtium officinale</i>	x
					<i>Rapistrum rugosum</i>	
					<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	
2824BD	2825AC	2824DB	2824BC	13	<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	
					<i>Brassica</i> sp.	x
					<i>Coronopus integrifolius</i>	x
					<i>Erucastrum griquense</i>	x
					<i>Heliophila affinis</i>	x
					<i>Heliophila arenaria</i> var. <i>arenaria</i>	x
					<i>Heliophila suavisissima</i>	x
					<i>Heliophila trifurca</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium ruderales</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium irio</i>	x
2824CA	2824AD	2823DD	2824AC	6	<i>Coronopus integrifolius</i>	x
					<i>Erucastrum griquense</i>	x
					<i>Lepidium desertorum</i>	x
					<i>Lepidium myriocarpum</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Rapistrum rugosum</i>	x
2824CB	2824DA	2824BC	2824CC	7	<i>Coronopus integrifolius</i>	x
					<i>Coronopus</i> sp.	x
					<i>Erucastrum griquense</i>	x
					<i>Heliophila minima</i>	x
					<i>Lepidium desertorum</i>	
					<i>Lepidium</i> sp.	x
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	x
2824CC	2824CD	2824CB	2824CA	3	<i>Coronopus</i> sp.	
					<i>Heliophila minima</i>	
					<i>Heliophila suavisissima</i>	x
					<i>Lepidium desertorum</i>	x
					<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	x
2824CD	2824CC	2824DC	2824CB	6	<i>Coronopus</i> sp.	x
					<i>Heliophila minima</i>	x
					<i>Heliophila suavisissima</i>	
					<i>Lepidium africanum</i>	x
					<i>Lepidium desertorum</i>	x
					<i>Lepidium ruderales</i>	x
					<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2824DA	2824CB	2824DC	2824CD	6	<i>Erucastrum griquense</i>	
					<i>Heliophila minima</i>	x
					<i>Heliophila suavisissima</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Lepidium africanum</i>	x
					<i>Lepidium desertorum</i>	x
					<i>Lepidium ruderae</i>	x
					<i>Lepidium</i> sp.	
					<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
2824DB	2824DA	2825CA	2824DD	6	<i>Brassica</i> sp.	
					<i>Coronopus integrifolius</i>	
					<i>Diplotaxis muralis</i>	x
					<i>Erucastrum griquense</i>	x
					<i>Heliophila affinis</i>	
					<i>Heliophila arenaria</i> var. <i>arenaria</i>	
					<i>Heliophila suavisissima</i>	
					<i>Heliophila trifurca</i>	
					<i>Lepidium africanum</i>	
					<i>Lepidium ruderae</i>	
					<i>Lepidium</i> sp.	x
					<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	
					<i>Sisymbrium capense</i>	
					<i>Sisymbrium irio</i>	
					<i>Sisymbrium</i> sp.	x
2824DC	2824DA	2824CD	2824DD	7	<i>Coronopus integrifolius</i>	x
					<i>Diplotaxis muralis</i>	x
					<i>Erucastrum griquense</i>	x
					<i>Heliophila minima</i>	
					<i>Heliophila suavisissima</i>	x
					<i>Lepidium africanum</i>	
					<i>Lepidium ruderae</i>	
					<i>Lepidium</i> sp.	x
					<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	
					<i>Sisymbrium</i> sp.	x
2824DD	2824DB	2825CA	2824DA	11	<i>Brassica</i> sp.	x
					<i>Coronopus integrifolius</i>	
					<i>Diplotaxis muralis</i>	
					<i>Erucastrum griquense</i>	
					<i>Heliophila affinis</i>	x
					<i>Heliophila arenaria</i> var. <i>arenaria</i>	x
					<i>Heliophila suavisissima</i>	
					<i>Heliophila trifurca</i>	x
					<i>Lepidium africanum</i>	
					<i>Lepidium ruderae</i>	x
					<i>Lepidium</i> sp.	x
					<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium irio</i>	x
					<i>Sisymbrium</i> sp.	
2825AA	2825AB	2824BD	2825AC	2	<i>Coronopus integrifolius</i>	x
					<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	x
2825AB	2825AA	2825AC	2725CD	1	<i>Coronopus integrifolius</i>	x
2825AC	2824BD	2825CA	2825AB	3	<i>Coronopus integrifolius</i>	
					<i>Erucastrum griquense</i>	x
					<i>Rorippa fluviatilis</i> var. <i>caledonica</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
2825AD	2825BA	2825CB	2825BC	0	None	
2825BA	2825BB	2825AD	2825BC	0	None	
2825BB	2825BA	2825BC	2826AC	1	<i>Brassica juncea</i>	x
2825BC	2825BA	2825AD	2825DA	0	None	
2825BD	2826AC	2826CA	2825CB	1	<i>Brassica juncea</i>	x
2825CA	2824DB	2824DD	2825AC	13	<i>Erucastrum griquense</i>	
					<i>Brassica</i> sp.	x
					<i>Coronopus integrifolius</i>	x
					<i>Diplotaxis muralis</i>	x
					<i>Heliophila affinis</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Heliophila arenaria</i> var. <i>arenaria</i>	x
					<i>Heliophila suavisissima</i>	x
					<i>Heliophila trifurca</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium ruderae</i>	x
					<i>Rorippa fluvialis</i> var. <i>fluvialis</i>	
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium irio</i>	x
					<i>Sisymbrium</i> sp.	x
2825CB	2825CA	2825BC	2825DA	0	None	
2825CC	2824DD	2925AA	2924BB	6	<i>Coronopus integrifolius</i>	x
					<i>Diplotaxis muralis</i>	x
					<i>Erucastrium griquense</i>	x
					<i>Heliophila suavisissima</i>	x
					<i>Lepidium africanum</i>	x
					<i>Sisymbrium</i> sp.	x
2825CD	2825CC	2825CB	2825DC	0	<i>Erucastrium strigosum</i>	
2825DA	2825CB	2825BC	2825DB	0	None	
2825DB	2825DA	2825BC	2825BD	0	None	
2825DC	2825DD	2925BB	2925BA	1	<i>Coronopus integrifolius</i>	x
2825DD	2826CC	2925BB	2825DC	2	<i>Coronopus integrifolius</i>	
					<i>Lepidium bonariense</i>	x
					<i>Nasturtium officinale</i>	x
2826AA	2826AB	2825BB	2826AD	0	<i>Lepidium</i> sp.	
2826AB	2826AA	2826BA	2826AD	1	<i>Lepidium</i> sp.	x
2826AC	2825BD	2826CA	2825BB	0	<i>Brassica juncea</i>	
2826AD	2826AA	2826AB	2826BA	1	<i>Lepidium</i> sp.	x
2826BA	2826AB	2826BB	2726DD	1	<i>Lepidium africanum</i>	x
2826BB	2726DD	2826BA	2826BC	1	<i>Lepidium africanum</i>	x
2826BC	2826BB	2826BA	2826DA	0	None	
2826BD	2826DA	2826DB	2827AA	0	None	
2826CA	2826AC	2825BD	2825DB	1	<i>Brassica juncea</i>	x
2826CB	2826DA	2826AD	2826BC	0	<i>Rorippa fluvialis</i> var. <i>caledonica</i>	
2826CC	2825DD	2826CD	2925BB	17	<i>Coronopus integrifolius</i>	x
					<i>Erucastrium austroafricanum</i>	x
					<i>Erucastrium griquense</i>	x
					<i>Erucastrium strigosum</i>	x
					<i>Heliophila minima</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Heliophila</i> sp.	x
					<i>Heliophila suavisissima</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	
					<i>Lepidium schlechteri</i>	x
					<i>Lepidium</i> sp.	x
					<i>Nasturtium officinale</i>	
					<i>Raphanus raphanistrum</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium orientale</i>	x
					<i>Sisymbrium thellungii</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2826CD	2926BA	2826DC	2826DA	0	<i>Coronopus integrifolius</i>	
					<i>Erucastrium austroafricanum</i>	
					<i>Erucastrium griquense</i>	
					<i>Erucastrium strigosum</i>	
					<i>Heliophila minima</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Heliophila</i> sp.	
					<i>Heliophila suavisissima</i>	
					<i>Lepidium africanum</i>	
					<i>Lepidium schlechteri</i>	
					<i>Lepidium</i> sp.	
					<i>Nasturtium officinale</i>	
					<i>Raphanus raphanistrum</i>	
					<i>Rapistrum rugosum</i>	
					<i>Sisymbrium capense</i>	

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Sisymbrium orientale</i>	
					<i>Sisymbrium thellungii</i>	
					<i>Sisymbrium turczaninowii</i>	
2826DA	2826DC	2826CD	2826BC	18	<i>Coronopus integrifolius</i>	x
					<i>Erucastrium austroafricanum</i>	x
					<i>Erucastrium griquense</i>	x
					<i>Erucastrium strigosum</i>	x
					<i>Heliophila minima</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Heliophila sp.</i>	x
					<i>Heliophila suavisissima</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium schlechteri</i>	x
					<i>Lepidium sp.</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium orientale</i>	x
					<i>Sisymbrium thellungii</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2826DB	2826BD	2826DA	2826BC	0	None	
2826DC	2826DA	2826CB	2826CD	17	<i>Coronopus integrifolius</i>	x
					<i>Erucastrium austroafricanum</i>	x
					<i>Erucastrium griquense</i>	x
					<i>Erucastrium strigosum</i>	x
					<i>Heliophila minima</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Heliophila sp.</i>	x
					<i>Heliophila suavisissima</i>	
					<i>Lepidium africanum</i>	x
					<i>Lepidium schlechteri</i>	x
					<i>Lepidium sp.</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluiatilis</i> var. <i>caledonica</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium orientale</i>	x
					<i>Sisymbrium thellungii</i>	x
					<i>Sisymbrium turczaninowii</i>	
2826DD	2826DB	2827CA	2926BB	3	<i>Capsella bursa-pastoris</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Sisymbrium capense</i>	x
2827AA	2727CD	2827AB	2826BD	0	None	
2827AB	2727CD	2727DC	2827AA	0	None	
2827AC	2827AA	2827CA	2827AD	2	<i>Erucastrium strigosum</i>	
					<i>Capsella bursa-pastoris</i>	x
					<i>Heliophila carnosa</i>	
					<i>Lepidium bonariense</i>	
					<i>Raphanus raphanistrum</i>	x
					<i>Sisymbrium capense</i>	
2827AD	2827AC	2827BC	2827CA	7	<i>Capsella bursa-pastoris</i>	x
					<i>Erucastrium strigosum</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Sisymbrium capense</i>	x
2827BA	2827AB	2727DC	2727CD	0	None	
2827BB	2828AA	2827BC	2827BD	3	<i>Heliophila carnosa</i>	x
					<i>Lepidium africanum</i>	x
					<i>Lepidium bonariense</i>	
					<i>Lepidium schinzii</i>	x
2827BC	2827BD	2827BB	2827CB	2	<i>Lepidium africanum</i>	
					<i>Lepidium bonariense</i>	x
					<i>Lepidium schinzii</i>	x
2827BD	2827DA	2827DB	2828AA	6	<i>Erucastrium austroafricanum</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Heliophila carnosa</i>	x
					<i>Heliophila</i> sp.	x
					<i>Heliophila suavisissima</i>	x
					<i>Lepidium schinzii</i>	
					<i>Lepidium</i> sp.	x
					<i>Sisymbrium turczaninowii</i>	x
2827CA	2827AC	2826DD	2827CC	6	<i>Capsella bursa-pastoris</i>	
					<i>Erucastrium strigosum</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila minima</i>	x
					<i>Heliophila suavisissima</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Raphanus raphanistrum</i>	
					<i>Sisymbrium capense</i>	
2827CB	2827BC	2827AD	2827CD	5	<i>Lepidium africanum</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Sisymbrium capense</i>	x
2827CC	2926BB	2827CB	2827CA	2	<i>Erucastrium strigosum</i>	
					<i>Capsella bursa-pastoris</i>	x
					<i>Heliophila carnosa</i>	
					<i>Heliophila minima</i>	
					<i>Heliophila suavisissima</i>	
					<i>Lepidium transvaalense</i>	
					<i>Raphanus raphanistrum</i>	x
					<i>Sisymbrium capense</i>	
2827CD	2827DA	2927AB	2927BA	2	<i>Heliophila suavisissima</i>	x
					<i>Lepidium schinzii</i>	
					<i>Lepidium transvaalense</i>	
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	
					<i>Sisymbrium capense</i>	
					<i>Sisymbrium turczaninowii</i>	x
2827DA	2827BD	2827CD	2827DD	8	<i>Brassica juncea</i>	x
					<i>Erucastrium austroafricanum</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila suavisissima</i>	
					<i>Lepidium bonariense</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium turczaninowii</i>	
2827DB	2827DD	2827DC	2828CC	6	<i>Erucastrium austroafricanum</i>	
					<i>Brassica juncea</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila digitata</i>	x
					<i>Heliophila</i> sp.	
					<i>Heliophila suavisissima</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium</i> sp.	
					<i>Sisymbrium capense</i>	x
2827DC	2927AB	2827DA	2827DD	5	<i>Brassica juncea</i>	x
					<i>Erucastrium austroafricanum</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila</i> sp.	
					<i>Heliophila suavisissima</i>	
					<i>Lepidium bonariense</i>	x
					<i>Sisymbrium capense</i>	
					<i>Sisymbrium turczaninowii</i>	x
2827DD	2827DA	2827DB	2828CC	5	<i>Brassica juncea</i>	
					<i>Erucastrium austroafricanum</i>	
					<i>Heliophila carnosa</i>	
					<i>Heliophila digitata</i>	x
					<i>Heliophila</i> sp.	x
					<i>Heliophila suavisissima</i>	x
					<i>Lepidium bonariense</i>	

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Lepidium</i> sp.	x
					<i>Sisymbrium turczaninowii</i>	x
2828AA	2828AB	2728CD	2827BD	11	<i>Erucastrium austroafricanum</i>	x
					<i>Heliophila carnosa</i>	
					<i>Lepidium schinzii</i>	x
					<i>Lepidium schlechteri</i>	x
					<i>Lepidium trifurcum</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2828AB	2828BA	2828AA	2728CD	2	<i>Erucastrium austroafricanum</i>	
					<i>Heliophila carnosa</i>	
					<i>Lepidium schinzii</i>	
					<i>Lepidium schlechteri</i>	
					<i>Lepidium trifurcum</i>	x
					<i>Nasturtium officinale</i>	x
					<i>Raphanus raphanistrum</i>	
					<i>Rapistrum rugosum</i>	
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	
					<i>Rorippa nudiuscula</i>	
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	
					<i>Sisymbrium turczaninowii</i>	
2828AC	2828CA	2828AD	2827DB	9	<i>Erucastrium austroafricanum</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila</i> sp.	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium</i> sp.	x
					<i>Raphanus raphanistrum</i>	x
					<i>Sisymbrium thellungii</i>	x
					<i>Turritis glabra</i>	x
2828AD	2828BC	2828CA	2828AC	11	<i>Diplotaxis muralis</i>	x
					<i>Erucastrium strigosum</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Heliophila</i> sp.	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Sisymbrium thellungii</i>	x
					<i>Sisymbrium turczaninowii</i>	x
					<i>Turritis glabra</i>	x
2828BA	2828AB	2828BB	2728DC	10	<i>Erucastrium austroafricanum</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium schlechteri</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rapistrum rugosum</i>	x
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium burchellii</i> var. <i>burchellii</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2828BB	2729CC	2828BA	2828BD	0	None	
2828BC	2828AD	2828BD	2828CB	1	<i>Diplotaxis muralis</i>	
					<i>Erucastrium strigosum</i>	
					<i>Heliophila carnosa</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Heliophila</i> sp.	
					<i>Lepidium schinzii</i>	
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium turczaninowii</i>	
2828BD	2828BC	2828DB	2829AA	12	<i>Diplotaxis muralis</i>	x
					<i>Cardamine africana</i>	x
					<i>Erucastrium strigosum</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Heliophila carnosa</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Heliophila</i> sp.	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium transvaalense</i>	x
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa nudiuscula</i>	x
					<i>Sisymbrium capense</i>	x
					<i>Sisymbrium turczaninowii</i>	x
2828CA	2828AC	2828AD	2828CC	1	<i>Heliophila carnosa</i>	
					<i>Heliophila digitata</i>	x
					<i>Lepidium bonariense</i>	
					<i>Lepidium schinzii</i>	
					<i>Raphanus raphanistrum</i>	
					<i>Sisymbrium thellungii</i>	
					<i>Turritis glabra</i>	
2828CB	2828CD	2828CA	2828BC	8	<i>Diplotaxis muralis</i>	x
					<i>Erucastum strigosum</i>	x
					<i>Heliophila carnosa</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Heliophila</i> sp.	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium schinzii</i>	
					<i>Raphanus raphanistrum</i>	x
					<i>Rorippa nudiuscula</i>	
					<i>Sisymbrium thellungii</i>	x
					<i>Sisymbrium turczaninowii</i>	x
					<i>Turritis glabra</i>	x
2828CC				–	<i>Heliophila digitata</i>	
2828DA				–	<i>Erucastum strigosum</i>	
					<i>Heliophila carnosa</i>	
					<i>Heliophila minima</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Heliophila</i> sp.	
					<i>Heliophila suavisissima</i>	
					<i>Lepidium bonariense</i>	
					<i>Lepidium schinzii</i>	
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	
2828DB	2828BC	2828DA	2828DD	8	<i>Cardamine africana</i>	
					<i>Diplotaxis muralis</i>	x
					<i>Erucastum strigosum</i>	x
					<i>Heliophila carnosa</i>	
					<i>Heliophila minima</i>	x
					<i>Heliophila rigidiuscula</i>	
					<i>Heliophila</i> sp.	x
					<i>Heliophila suavisissima</i>	x
					<i>Lepidium bonariense</i>	x
					<i>Lepidium schinzii</i>	x
					<i>Lepidium transvaalense</i>	
					<i>Raphanus raphanistrum</i>	
					<i>Rorippa fluviatilis</i> var. <i>fluviatilis</i>	x
					<i>Rorippa nudiuscula</i>	
					<i>Sisymbrium capense</i>	
					<i>Sisymbrium turczaninowii</i>	
2829AA	2829AB	2828BD	2829AC	5	<i>Capsella bursa-pastoris</i>	x
					<i>Diplotaxis muralis</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila minima</i>	x
					<i>Heliophila rigidiuscula</i>	x
2829AB	2829AA	2829AC	2729CC	5	<i>Capsella bursa-pastoris</i>	x
					<i>Diplotaxis muralis</i> .	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila minima</i>	x
					<i>Heliophila rigidiuscula</i>	x
2829AC	2829AD	2829AA	2829AB	4	<i>Capsella bursa-pastoris</i>	
					<i>Brassica elongata</i> subsp. <i>elongata</i>	x
					<i>Cardamine flexuosa</i>	x
					<i>Cardamine impatiens</i>	x

QDGC of study area	Most similar QDGC			n species added	Species	Added (x)
	1	2	3			
					<i>Diplotaxis muralis</i>	
					<i>Heliophila carnosa</i>	
					<i>Heliophila minima</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Nasturtium officinale</i>	x
2829AD				–	<i>Brassica elongata</i> subsp. <i>elongata</i>	
					<i>Cardamine flexuosa</i>	
					<i>Cardamine impatiens</i>	
					<i>Heliophila carnosa</i>	
					<i>Heliophila rigidiuscula</i>	
					<i>Nasturtium officinale</i>	
2829BA	2729DC	2829AD	2829AD	7	<i>Brassica elongata</i> subsp. <i>elongata</i>	x
					<i>Cardamine flexuosa</i>	x
					<i>Cardamine impatiens</i>	x
					<i>Heliophila carnosa</i>	x
					<i>Heliophila cornuta</i>	x
					<i>Heliophila rigidiuscula</i>	x
					<i>Nasturtium officinale</i>	x