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Species richness and diversity of a terrestrial insular environment: serpentine of the Barberton Greenstone Belt, South Africa

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The serpentinite outcrops of the Barberton Greenstone Belt are considered to be amongst the oldest in the world and have been exposed for over 50 million years. Of the 30 large outcrops in eastern Mpumalanga, South Africa, seven were selected for detailed comparison of species richness and diversity with those of surrounding non-serpentine vegetation. Various non-parametric species richness estimators were calculated to predict the species richness of each site and these indicated that four serpentine outcrops have higher and three have lower species richness than the surrounding non-serpentine areas. The Shannon, Simpson's and Fisher's alpha indices of diversity were calculated for each site on and off serpentine and compared. The diversity values calculated show similar patterns to those of the species richness estimates. Significant differences in species composition between floras on and off serpentine were estimated using Sørenson's Index of similarity. The serpentine outcrops of the Barberton Greenstone Belt show relatively high diversity when compared to some other serpentine outcrops around the world.

Key words: Diversity indices, *EstimateS*, Modified-Whittaker plots, serpentine, species accumulation curves, species richness estimation.

INTRODUCTION

The diversification of habitats that can flow from differences in substrates such as limestones, dolomite, shale, gypsum and serpentine becomes the stimulus for evolutionary diversification by speciation. These azonal substrates have been found to harbour unique plant associations, including endemic species, and foster morphological and physiological modifications of plants as well as unusual distributions of taxa (Kruckeberg, 1986). Serpentine soils often support a flora distinct from the surrounding vegetation due to phytotoxic concentrations of heavy metals such as nickel and chromium and high ratios of magnesium to calcium (Roberts and Proctor, 1992). In addition, serpentine outcrops support endemics that usually occur in a few small populations and are confined to single or few localities (Kruckeberg and Rabinowitz, 1985), which have conservation and scientific value. A 'Resolution' passed by delegates of The First International Conference on Serpentine Ecology held in 1991 supports the conservation of the vegetation of serpentine areas worldwide (Kruckeberg, 1992).

These serpentine outcrops provide a classic system for understanding the origins and maintenance of plant diversity. It is usually predicted that the insularity of serpentine outcrops will result in low plant species richness (α diversity) but high ß diversity, that is, spatial

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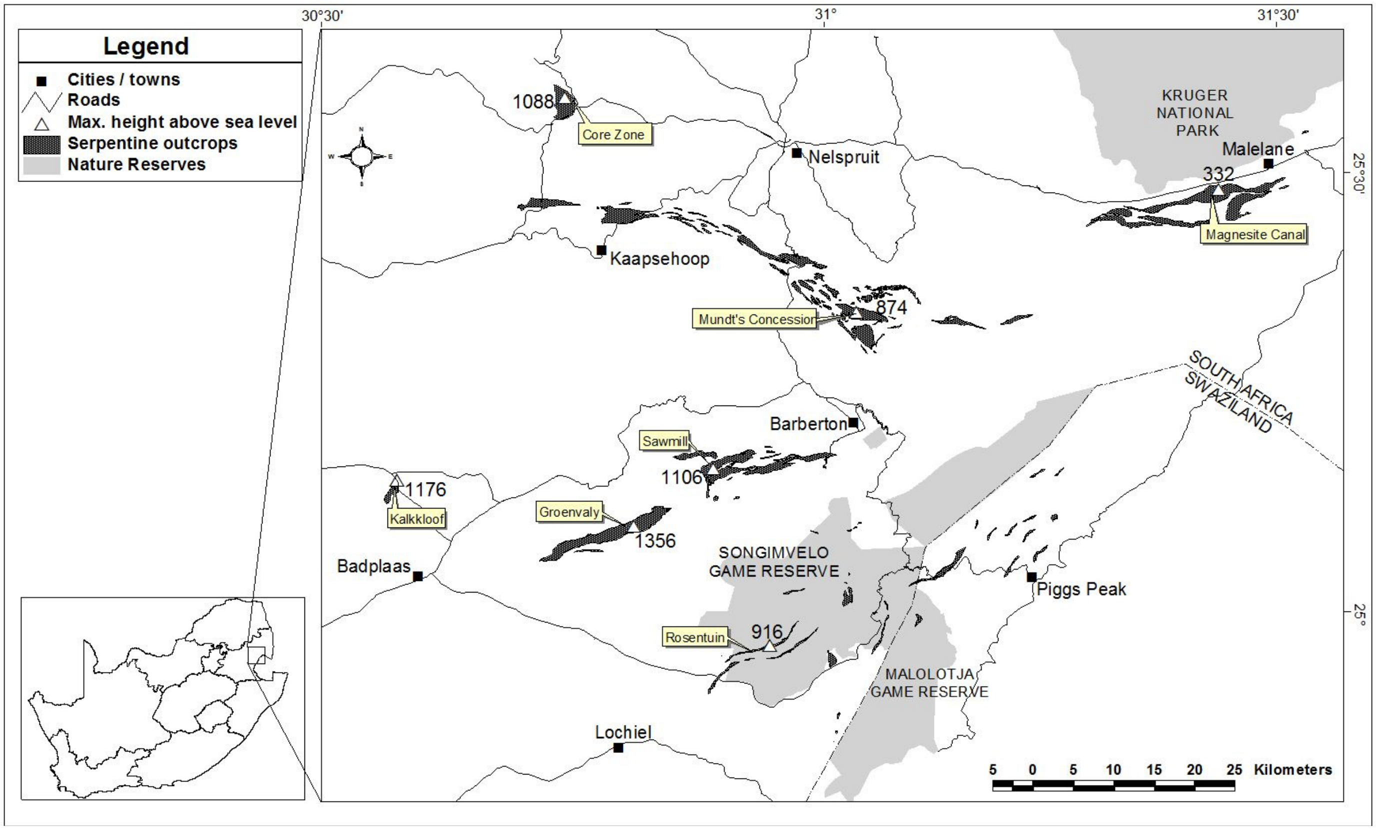

Figure 1. Map indicating serpentine outcrops of the Barberton Greenstone Belt in Mpumulanga, South Africa. Survey sites are identified by callout labels. Map was prepared using data provided by the Chief Directorate: Surveys and Mapping, Department of Land Affairs, Republic of South Africa.

variation in species composition among regions (Harrison and Inouye, 2002). In contrast, the serpentine of the Siskiyou and Wenatchee Mountains in the United States of America were shown to support higher species richness than the surrounding non serpentine areas (Proctor and Woodell, 1975). The high species richness recorded on the serpentine outcrops of South Central Africa (Wild and Bradshaw 1977) suggests that have been exposed sufficiently long to re-establish diversity following the geological changes associated with this exposure (Brown 1988). It is suggested that the Barberton Greenstone Belt is between 3.3 and 3.4 billion years old and is considered to be the second oldest greenstone belt after the Isua Greenstone Belt in Greenland (Condie, 1981). The rocks of the Barberton Greenstone Belt are thought to have been exposed for about 50 million years (Anhaeusser *pers. comm.*). This extended time of exposure could have resulted in the establishment of high diversity on the serpentine sites of the Barberton Greenstone Belt (Brooks, 1987). The serpentines in central Cuba are believed to have been exposed for only around 1 m.y. (Reeves et al., 1996) and those of California were exposed between the Late Pliocene to Early Quaternary (Raven and Axelrod, 1978).

The term ‘serpentine’ is often applied to ultramafic rocks in general, but in this study it is used in its strict sense and applied to rocks derived from serpentinite. There are about 30 large (that is, >1 km^2) serpentine outcrops, with the largest about 19 km^2, and many smaller outcrops (from 0.1 km^2) in eastern Mpumalanga (Figure 1), which form part of the Barberton Greenstone Belt (Ward, 2000). These outcrops are located in an inverted equilateral triangle centred on Barberton extending to Malelane in the east and to Badplaas in the south. Some outcrops are separated from others by up to 20 km (Balkwill et al., 1997). The outcrops occur in mountainous areas and are heterogeneous in altitude, slope, soil depth and other topographic features. The serpentine vegetation falls within the Mixed Lowveld Bushveld, Sour Lowveld Bushveld and North-eastern Mountain Grassland vegetation types (Low and Rebelo, 1996). A more recent classification recognises the unique character of the serpentine vegetation of the Barberton Greenstone Belt and labels it as Barberton Serpentine Sourveld (Mucina and Rutherford, 2006). In addition, the vegetation of these serpentine outcrops forms part of the Barberton Centre of Endemism (van Wyk and Smith, 2001).

As on other serpentine outcrops around the world, the flora of the Barberton Greenstone Belt seems to be distinct from the surrounding vegetation, although this has yet to be quantified. These outcrops do, however, support a large number of relatively rare endemic species most of which have red data status and thus are of conservation interest (Williamson and Balkwill, 2006).

Ecologists have long been intrigued by patterns of spatial and temporal variation in the diversity of plants and animals, and there are now a large array of indices and models that have been developed for quantifying species diversity (Magurran, 1988). Determining species diversity for selected areas and an understanding of the factors that regulate species diversity are central to the science of conservation (Spellerberg, 1992; Cowling, 1983). Species diversity consists of two components, namely: species richness, which is the number of species present, and evenness or the relative abundances of species. A "diversity index" is thus a single statistic that incorporates both richness and evenness into a single value (Magurran, 2004). An area is said to have high diversity if it has many species and their abundances are fairly even. Diversity is low when there are few species with uneven abundances (George and Hanumara, 1989). Selecting an appropriate measure of diversity depends largely on the question the index is being used to answer, the component of diversity being measured and whether the index is simple to use and understand (Williams et al., 2005).

In most studies, the diversity of serpentine vegetation has been expressed in terms of endemic species richness only (Selvi, 2007) and the focus for determining the conservation value of serpentine outcrops has been in terms of the number of endemics and the percentage endemism (Brooks, 1987). Few authors have described the diversity of the vegetation of various serpentine outcrops and the factors affecting diversity, which is essential for determining conservation priorities for these outcrops. The species richness of serpentine outcrops in Tuscany (Italy) was used to identify centres of diversity and endemism and to evaluate the environmental factors that affect the abundance of these plant communities (Chiarucci et al., 2001). There is evidence that the species richness of the Tuscan serpentine outcrops decreases with increasing isolation of outcrops and decreasing surface area (Selvi 2007). These data were used to develop conservation programs for the serpentine outcrops in Tuscany (Italy). The diversity of plant species on ultramafics and nearby schist substrates in New Zealand was analysed to determine trends in diversity in relation to altitude (Bastow Wilson et al., 1990). The serpentine areas of California are considered to support one of the world's richest serpentine floras. The vegetation of the Californian serpentines shows higher diversity on the more continuous serpentine areas and lower diversity on the areas which are naturally more patchy (Harrison, 1997). The high endemic diversity of the Californian serpentine outcrops is determined by high precipitation and high soil Mg/Ca ratios (Grace et al., 2007).

Describing the species diversity of metallophytes (organisms with the ability to tolerate or avoid the toxic effects of metals) is essential to understand and conserve serpentine outcrops (Whiting et al., 2004). The serpentine vegetation of the Barberton Greenstone Belt is considered to have conservation value in an area that is heavily utilised for *Eucalyptus* and pine plantations (Balkwill et al., 1997) and thus for effective conservation and environmental management the baseline species richness and diversity needs to be described.

Numerous theories have been advanced to account for patterns of species diversity across edaphic boundaries such as that of serpentine outcrops and their surrounding vegetation. One is that which edaphic 'islands' have re-established diversity after a sufficiently long period after perturbation (Brown, 1988). In contrast it has been suggested that the insularity of serpentine outcrops will result in low plant species α diversity (Harrison and Inouye, 2002). As the first part of a wider study to determine whether serpentine areas have high conservation value in terms of diversity, this paper attempts to test the hypothesis that the vegetation of the serpentine outcrops of the Barberton Greenstone Belt has higher species richness and diversity than the adjacent non-serpentine areas. The differences in species richness and diversity between serpentine and adjacent non-serpentine areas of the Barberton Greenstone Belt are quantified using a range of applicable richness and diversity indices. These differences would determine whether the vegetation of the Barberton Greenstone Belt supports either of the predictions made regarding species diversity patterns caused by edaphic variation. This paper also aims to determine whether all serpentine outcrops of the Barberton Greenstone Belt have similar plant species richness and diversity and to provide preliminary data to help determine the factors contributing to any measured differences in diversity. The species richness and diversity values calculated could provide a strong case for including serpentine vegetation in the planning for conservation of the vegetation of the southern portion of Mpumalanga Province. Land-use decisions are most often based on variation and species diversity on the scale of landscapes or 'park-sized' units and have significant impact on the long-term future of biodiversity (Colwell and Coddington, 1994). Lastly, this paper aims to compare the diversity of the serpentine sites of the Barberton Greenstone Belt to other serpentine sites around the world.

MATERIALS AND METHODS

There are approximately 30 large serpentine outcrops in the Barberton Greenstone Belt surrounded by several very small outcrops. Of the large ones, seven were selected to be studied in detail. This selection was made so that presumably the full range of variation in terms of size, altitude and position is accounted for and excluded the Swaziland outcrops and other outcrops which have previously been studied in detail (McCallum, 2006; Changwe and Balkwill, 2003; Williamson, 1994).

Four modified-Whittaker plots of 1000 m^2 with ten 1 m^2, two 10 m^2 and a single 100 m^2 subplot within the largest plot (Stohlgren et al., 1995), were positioned on each serpentine site and adjacent non-serpentine area. These plots were positioned at the base, on the mid-slopes and crests of hills. This provided data from 56 modified-Whittaker plots for analysis. Within each plot and subplot, the numbers of taxa present and their relative abundances were recorded.

Species richness

There are two main methods of expressing estimates of species richness. The first method computes numerical species richness, which is the number of species per specified number of individuals or biomass. The second method describes species density, which is the number of species per specified collection area or unit. For the purpose of comparison between serpentine vegetation and adjacent non-serpentine vegetation and to compare the species richness of the sampled serpentine outcrops to other areas, species richness has been calculated with both methods using the data collected in the Modified-Whittaker plots.

For the measurement and comparison of species richness, the use of taxon sampling or accumulation curves is recommended (Colwell and Coddington, 1994; Gotelli and Colwell, 2001) recommend. Public-domain software, EstimateS (Version 7.5, Colwell, 2005) was used to compute randomised species accumulation curves with 95% confidence intervals for each serpentine and adjacent non-serpentine site. To eliminate any variation in curve shape due to sampling error or heterogeneity among sampled units (Colwell and Coddington, 1994); EstimateS randomises the sample order and calculates the mean and standard deviation of $S(n)$ (S represents observed species richness and n the number of samples) computed for each value of n.

Determining species richness from samples invariably underestimates the total species richness of the plant communities, and the total enumeration of species richness within a large study area is generally not feasible (Chiarucci et al., 2003). Non-parametric estimates of species richness have been shown to approach the actual species richness values; however, they are still thought to underestimate total species richness (Chiarucci et al., 2003). EstimateS was used to calculate seven non-parametric estimators of total species richness. Non-parametric methods for estimating species richness were used in preference to fitting parametric models of relative abundance as the non-parametric methods require no assumption about community structure (Colwell and Coddington, 1994).

Chao 2 is simple estimator of absolute number of species in an assemblage, which is calculated on presence/absence data taking into consideration the distribution of species amongst samples (Chao 1984). Jackknife 1, a first-order jackknife estimator, employs the number of species that occur in only a single sample and can be calculated to include the number of species in two samples (Jackknife 2) (Magurran, 2004). The bootstrap estimator used, is based on the proportion of quadrats containing each species and requires only incidence data (Smith and van Belle 1984). Plotting the performance of the estimator on a species accumulation curve illustrates the rate at which new species are found (Magurran, 2004) and demonstrates the performance of the estimator and differences in relative abundance as sample size increases (Williams et al., 2007). Species richness estimators may form the basis of community comparison, providing a convincing asymptote is reached (Magurran, 2004).

The observed species richness calculated for each site was correlated with various physiographic factors using Multiple Regression Analysis to determine the factors affecting species richness. These factors include: area of outcrop (km^2); mean elevation (meters above sea level); the latitude of the outcrop; available nickel and chromium; and the calcium to magnesium ratio of the soil.

Species diversity

Indices of diversity or heterogeneity incorporate measures of species richness and evenness into a single value and are based on the proportional abundance of species in a sample (Magurran, 1988). Three diversity indices that is, Simpson's Index expressed as lambda (λ), the Shannon Index (H') and Fisher's Alpha, were selected to quantify and compare the diversity of the vegetation of the serpentine outcrops and the surrounding non-serpentine areas.

Simpson's Index is a diversity index which is weighted by abundances of the commonest species and measures the probability that two randomly selected individuals from a sample will belong to the same species (Magurran, 2004). A rarely cited function is $-\ln \lambda$, is preferred by Williams et al. (2005) as it does not represent a probability, but a single diversity statistic that increases as diversity increases and gives numbers of similar magnitude to those of the Shannon index (Magurran, 2004). Simpson's index of diversity was selected as it provides a good estimate of diversity at relatively small sample sizes, will rank assemblages consistently and confidence limits can be attached to it (Magurran, 1988).

The Shannon index (H') is sensitive to sample size (Magurran, 2004) as it assumes that individuals are randomly sampled from an infinitely large community and that all species are represented in the sample (Pielou, 1975). The Shannon index is, therefore, one of the information statistics which tend to emphasize the species richness component of diversity (Magurran, 2004) as it measures the average degree of 'uncertainty' in predicting to what species individuals chosen at random will belong. It was selected for this study due to the simplicity of calculating H' and its widespread use as a benchmark measure of biological diversity. Large values of H' (high diversity) indicate a greater uncertainty in correctly predicting the identity of the next species chosen at random.

Fisher's alpha (α) describes the relationship between the number of species and the number of individuals of those species with a logarithmic distribution. It represents a species abundance model that could shed light on the processes that determine the biological diversity of an assemblage (Magurran, 2004). Alpha is low when the number of species is low and, therefore, smaller samples have low values of α. The index is less affected by the abundance of the rarest or commonest species than either H' or λ respectively and depends more on the number of species of intermediate abundance.

In order to compare the diversity calculated for different assemblages statistically, Magurran (2004) suggests using a jackknifing method. This method involves producing a series of 'pseudovalues' and the means of these pseudovalues represent the best estimate of the diversity statistic. Approximate confidence limits can then be attached to the estimate. This was done to determine whether the diversity of each serpentine site is significantly different to that of the adjacent non-serpentine area.

RESULTS AND DISCUSSION

Species richness

The number of recorded species per specified collection area or species density, mostly represented as species per m^2, is a commonly used measure of species

Table 1. Number of species recorded in 1000 m^2 (0.1ha) for each Barberton Greenstone Belt serpentine outcrop and adjacent non serpentine plots including the Swaziland serpentine outcrops. For all sites the differences in species density between serpentine sites and their adjacent non-serpentine areas are not statistically different as P>0.05 (◆, data not available).

Site	Species density (no of species per 0.1ha)				t value and probability of difference
	Serpentine outcrops		Adjacent non-serpentine area		
	Mean ± SD	Range	Mean ± SD	Range	
Sawmill	74.5±6.6	67-83	78.0±10.8	66-92	t(cal) = -0.668, P>0.05
Rosentuin	71.8±13.4	55-87	69.8±6.6	64-78	t(cal) = 0.215, P>0.05
Mundt's	65.3±4.7	62-71	67.3±4.7	63-73	t(cal) = -0.627, P>0.05
Magnesite	73.3±7.5	67-82	54.0±9.4	44-66	t(cal) = 2.339, P>0.05
Kalkkloof	83.3±7.3	73-90	82.8±9.3	71-93	t(cal) = 0.099, P>0.05
Groenvaly	80.3±9.4	69 - 92	72.5±13.4	55 - 86	t(cal) = 0.847, P>0.05
Core Zone	74.8±5.1	70 - 82	84.3±5.7	79 - 92	t(cal) = -1.956, P>0.05
Dunbar[1]	29.0±4.3	◆	31.7±2.9	◆	t(cal) ◆, P>0.05
Forbes Reef [2]	81.7±5.6	◆	73.0±2.3	◆	t(cal) ◆, P>0.05
Malolotja[2]	76.3±11.2	◆	54.5±6.5	◆	t(cal) ◆ P>0.05
Motjane[2]	43.5±0.5	◆	59.0 (single)	◆	t(cal) ◆, P>0.05

[1]Changwe and Balkwill (2003). [2]McCallum (2006).

richness (Magurran, 1988). There is considerable variation in the mean number of species per 0.1ha between the selected sample sites, ranging from 29 to 65 species per 0.1 ha (Table 1). Although the number of species per 0.1 ha for four of the sites is shown to be considerably higher than that of the adjacent non-serpentine areas and for two of the sites the species number is considerably lower, these differences were found to be not significantly different ($P > 0.05$) when compared using paired T-tests. However, the Magnesite, Corezone, Malolotja and Motjane sites $P < 0.2$, which suggests a true biological difference.

Randomised species accumulation curves for species recorded in plots for each serpentine outcrop and the adjacent non-serpentine areas per sample are shown in Figure 2. The species accumulation curves in Figure 3 represent a comparison of species richness where the effect of density has been removed by pooling individuals of the same species. The species richness values were plotted together with the 95% confidence intervals for each curve as Colwell et al. (2004) conclude that where confidence levels do not overlap the differences in species richness are significant at $P<0.05$. The graphs of numerical species richness (S) for each site (Figure 3) indicate that sampling was slightly inadequate as some of the curves are approaching an asymptote, but most curves are increasing at a rate of 0.08 to 0.12 species per individual at the end of each curve. At this level of sampling, results show that the non-serpentine vegetation has higher species richness than the serpentine vegetation for two of the seven sites and only Groenvaly serpentine shows significantly higher species richness than the surrounding non-serpentine area ($P<0.05$). Although the species richness on and off serpentine of most sites is quite similar, the percentage of species shared is 33% or less for all sites (Table 2), indicating a large change in species composition across soil substrates.

The differences in the number of shared species on and off serpentine (Table 2) could be due to the different vegetation types surrounding the serpentine outcrops, and possibly show that grassland species are less able to colonise serpentine soils than bushveld species. The graphs in Figures 2 and 3 differ because the relative abundances (measured as the mean number of individuals per sample) are greater in the serpentine vegetation than the non-serpentine vegetation for six sites. The relative abundance for the serpentine of Core Zone is considerably lower (9.9 individuals per species) than the adjacent non-serpentine (12.7 individuals per species).

The dendrogram (Figure 4a) prepared from the Sorensøn's Similarity Index comparing species composition between serpentine sites (Table 3) groups outcrops in a way that correlates with geography and altitude to a large extent and

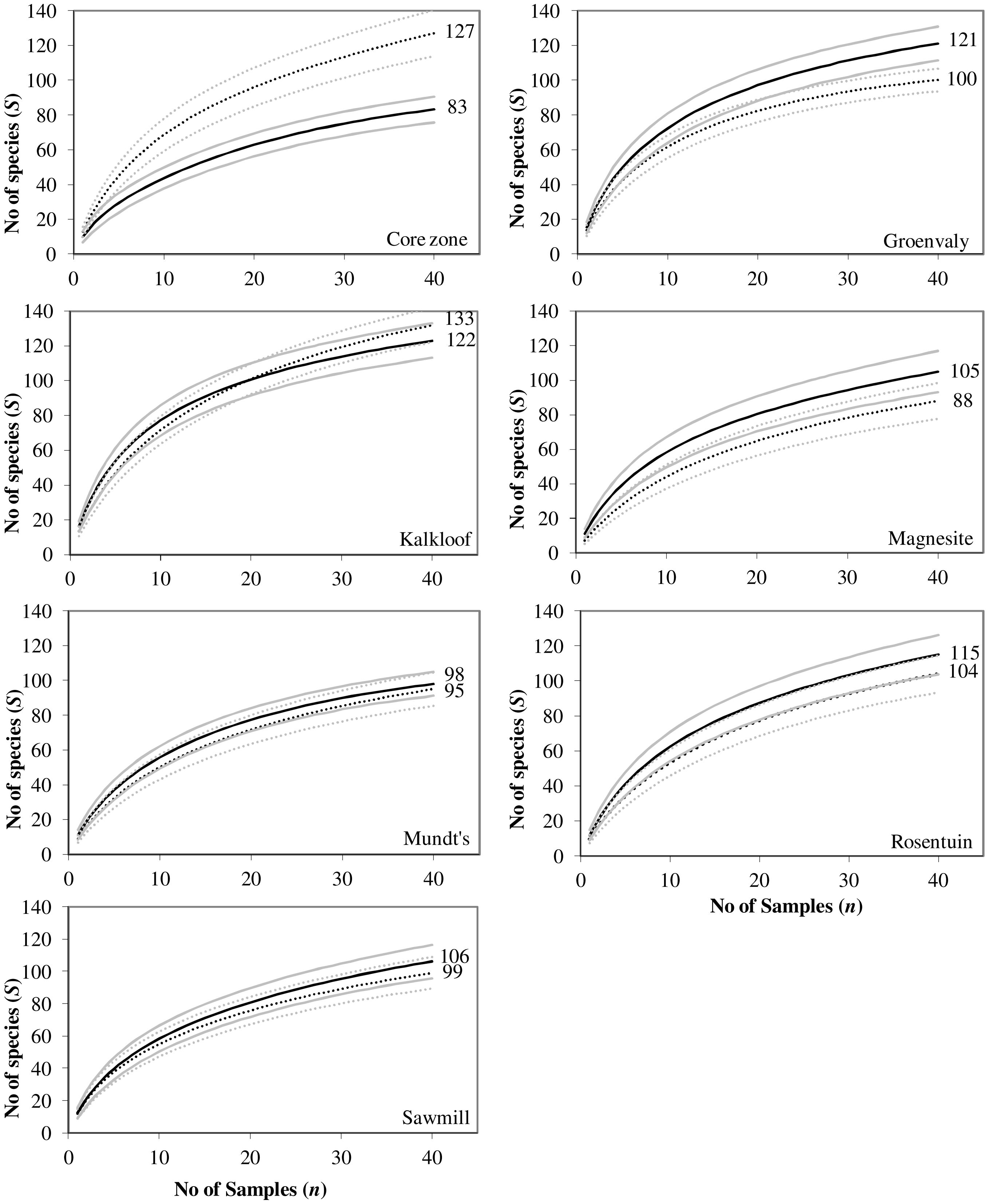

Figure 2. Observed species richness of selected serpentine sites (solid lines) and adjacent non-serpentine areas (broken lines) plotted against number of 1 m^2 samples. The overall value of S for the randomly pooled samples is labelled at the end of each curve and 95% confidence intervals are shown in grey. All graphs are scaled to the same x and y-axes values.

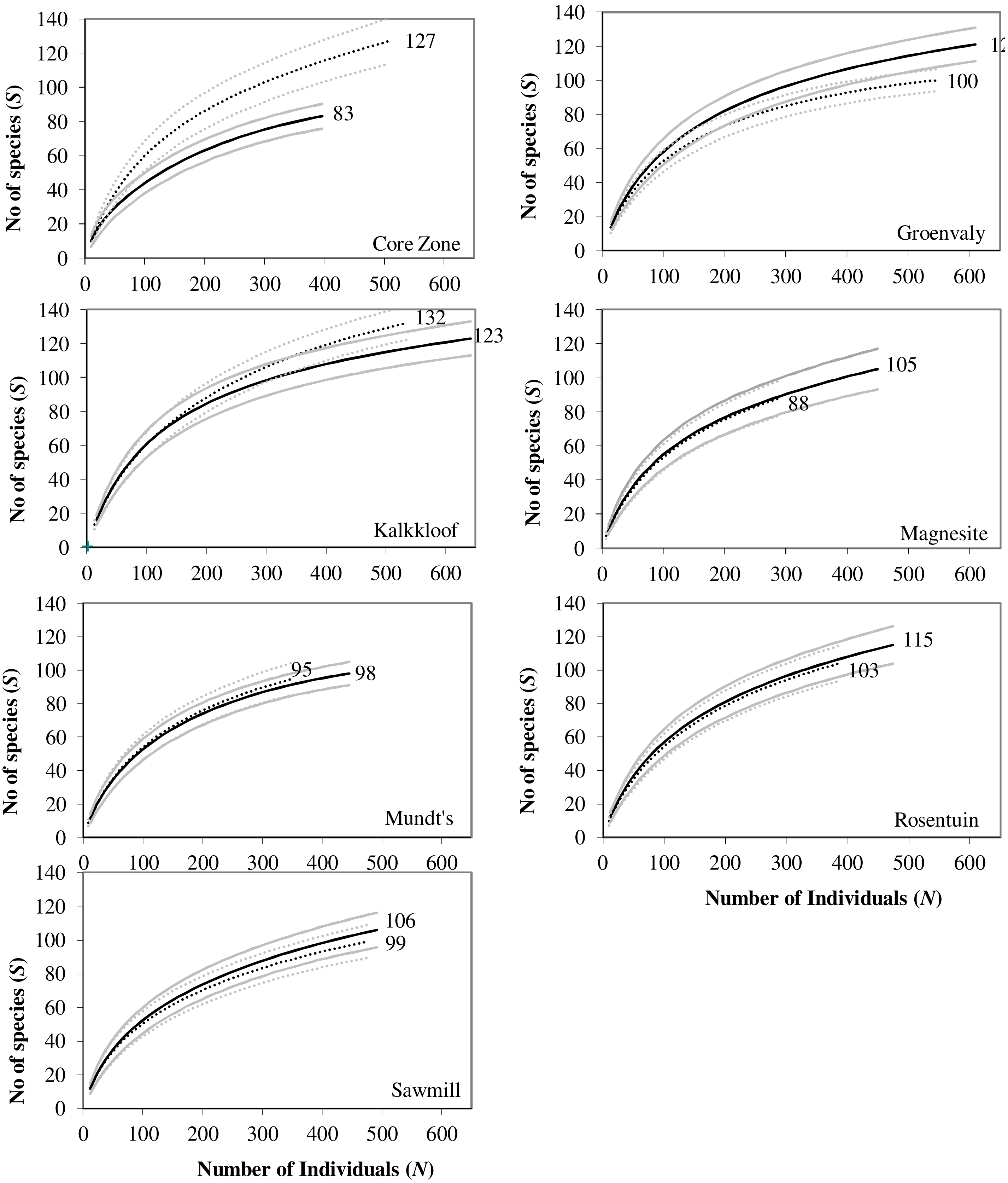

Figure 3. Observed species richness of selected serpentine sites (solid lines) and adjacent non-serpentine areas (broken lines) plotted against individual numbers (indicating sampling intensity). The overall value of S for the randomly pooled samples is labelled at the end of each curve and 95% confidence intervals are shown in grey. All graphs are scaled to the same x and y-axis values.

Table 2. Sørenson's coefficient of similarity for serpentine and non-serpentine areas for each site of the Barberton Greenstone Belt, expressed as percentages, correlated with the vegetation type within which the outcrop falls.

Site	% Similarity	Vegetation type (Low and Rebelo, 1996)	Vegetation type (Mucina and Rutherford, 2006)
ore-Zone	21.69	Sour Lowveld Bushveld	Legogote Sour Lowveld Bushveld
Groenvaly	26.42	Sour Lowveld Bushveld	Barberton Montane Grassland
Magnesite Canal	28.03	Mixed Lowveld Bushveld	Kaalrug Mountain Bushveld
Kalkkloof	28.18	North Eastern Mountain Grassland	KaNgwane Montane Grassland
Sawmill	28.93	Sour Lowveld Bushveld	Barberton Montane Grassland
Dunbar[1]	31.2	North Eastern Mountain Grassland	Swaziland Sour Bushveld
Mundt's Concession	33.03	Sour Lowveld Bushveld	Granite Lowveld
Rosentuin	33.33	North Eastern Mountain Grassland	Swaziland Sour Bushveld
Malolotja[2]	35.7	North Eastern Mountain Grassland	Barberton Montane Grassland
Forbes reef[2]	42.2	North Eastern Mountain Grassland	Barberton Montane Grassland
Motjane[2]	74.7	North Eastern Mountain Grassland	Barberton Montane Grassland

[1] Changwe and Balkwill (2003); [2] McCallum (2006).

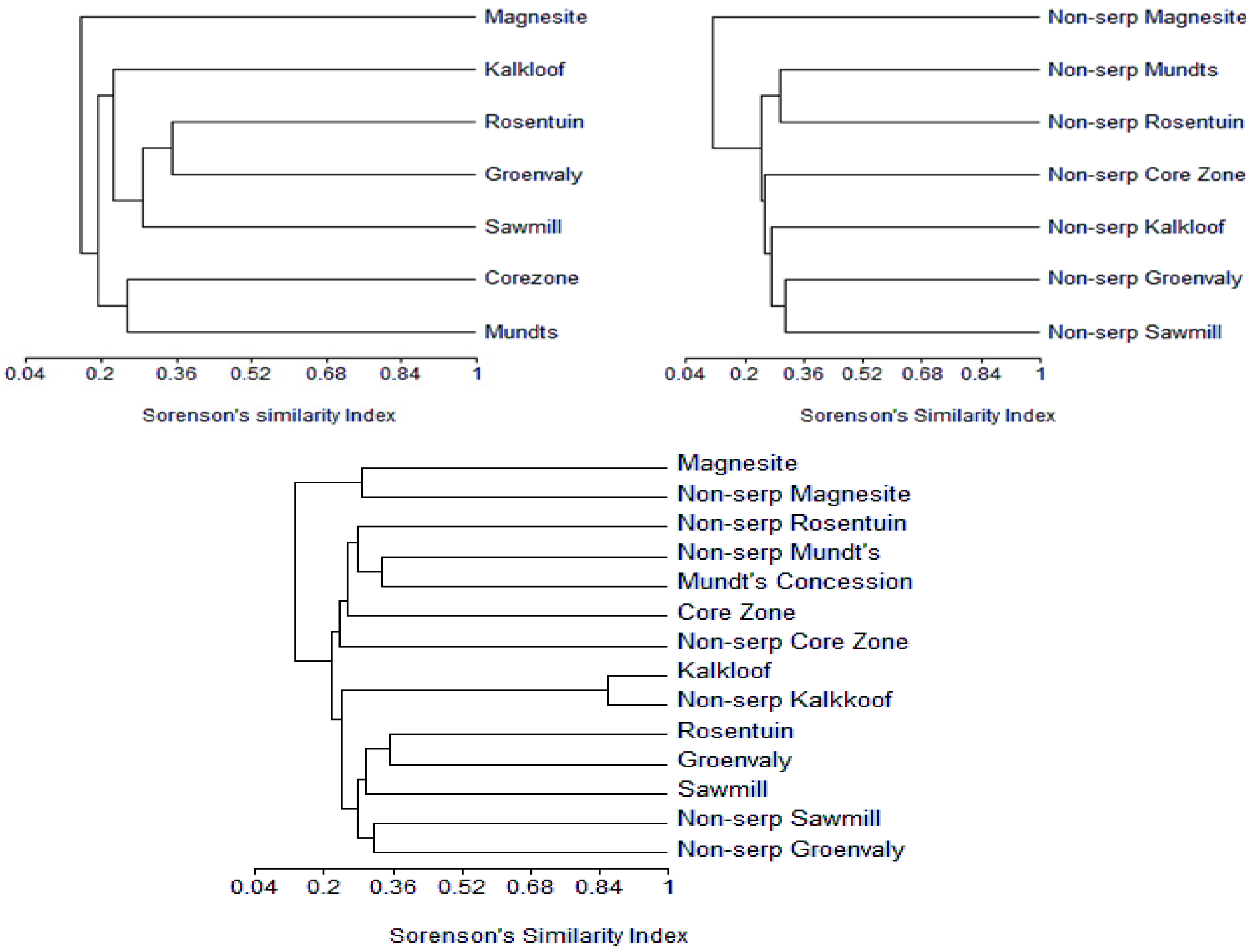

Figure 4 Dendrograms representing the Sørenson's coefficient measuring the similarity between sampled serpentine sites, the adjacent non-serpentine samples and a combination of both serpentine and non-serpentine samples. Dendrograms were drawn online using the UPGMA algorithm.

Table 3. Sørenson's coefficient of similarity measuring the degree of similarity between sampled serpentine sites, expressed as percentages.

Site	Core zone	Groenvaly	Kalkkloof	Magnesite Canal	Mundt's Concession	Rosentuin
Groenvaly	17.7	-				
Kalkkloof	18.3	25.4	-			
Magnesite	*14*	18.9	9.4	-		
Mundt's	25.4	27.5	15.7	21.1	-	
Rosentuin	17	**35**	24.9	15.9	22.5	-
Sawmill	16.2	34.1	17	*14*	21.1	23.3

indicates that Rosentuin and Groenvaly sites are most similar.

The high similarity between these two outcrops is possibly due to their proximity and that they seem to be part of the same group of outcrops (Figure 1). Although the Magnesite site has similar observed species richness to most of the other sites, it shares very few taxa with the other sites. This site is part of a spatially isolated group of outcrops that are found at a much lower altitude (Figure 1) than the other outcrops and are surrounded by a very different vegetation type (Table 2). The dendrogram representing the similarity between the non-serpentine samples (Figure 5b) also isolates the Magnesite sample from the other groups, but groups the other samples quite differently showing the Groenvaly sample most similar to the Sawmill sample.

When the data are combined, the dendrograms show that the serpentine sites that are geographically clustered are more similar in species composition to each other than to their adjacent non-serpentine vegetation and form two distinct groups of outcrops. The geographically isolated serpentine sites are seen to be more autochthanous. Of interest is that the Kalkloof serpentine and non-serpentine samples are the most similar to one another of any pair. This serpentine site has the lowest nickel and chromium levels of all other sites.

In many cases, observed species richness underestimates actual species richness as it is likely that not all species were sampled in the plots. Therefore, non-parametric methods, which estimate the lower bounds of predicted total species numbers, are used to estimate species richness (Colwell, 2005). The Chao and ICE estimators over-estimated species richness at the beginning of a sample (because they are strongly affected by the high number of singletons and rare species present in the first few samples) making them unstable initially and were therefore discounted. The second- and first-order Jack knife estimators (Jack 2 and Jack 1) consistently generate the highest estimates and the Bootstrap the lowest estimates for all samples (Figure 5). Jack 2 seems to approach an upper limit for the number of species likely to be found at each site and Bootstrap provides the lower-bound estimate of species richness. For six of the seven sites (excluding Mundt's Concession), the estimators tend to rise in parallel with the observed species accumulation curve and do not reach an asymptote, but show the same trends in terms of comparisons between serpentine and non-serpentine areas at each site.

The upper and lower bound predictors of expected species richness show the same trend, as the observed species richness, for the Groenvaly site with the non-serpentine curve having reached an asymptote but the serpentine curve still increasing slightly (Figure 5). This suggests that the actual difference in species richness may be even greater than measured here. The upper bound species richness estimator, Jack 2, representing the upper bound of species richness, predicts that differences between serpentine and non-serpentine are greater than that suggested by the observed species richness. It suggests significant difference ($P<0.5$) in five of the sites and biological difference ($P<0.02$) for the Rosentuin and Sawmill sites (Table 4).

Species diversity and evenness

The Simpson's, Shannon and Fisher's alpha diversity indices were used as they highlight different aspects of diversity in a plant community (Magurran, 2004). It was thus expected that the different indices used would possibly highlight differences between the serpentine and non-serpentine vegetation other than those shown by the species richness values. However, the species diversity indices calculated for each site show similar trends to those established by the species richness estimators that is, serpentine areas that show significantly higher species richness than non-serpentine areas also show significantly higher species diversity (Table 4).

The evenness (J') values are very similar for serpentine and non-serpentine areas, which suggests that the species richness component, of the diversity indices, contributes significantly to the differences between individual serpentine and non- serpentine areas and not their relative abundance based on the evenness (J') index. The Corezone site is one exception as the evenness (J') calculated for the non-serpentine area is significantly higher than that for the serpentine outcrop. This suggests that the non-serpentine vegetation here has more species that are equally abundant, that is, there is higher dominance of certain species on the serpentine outcrop. The evenness values for the other sites is contradicted by the differences in relative abundance

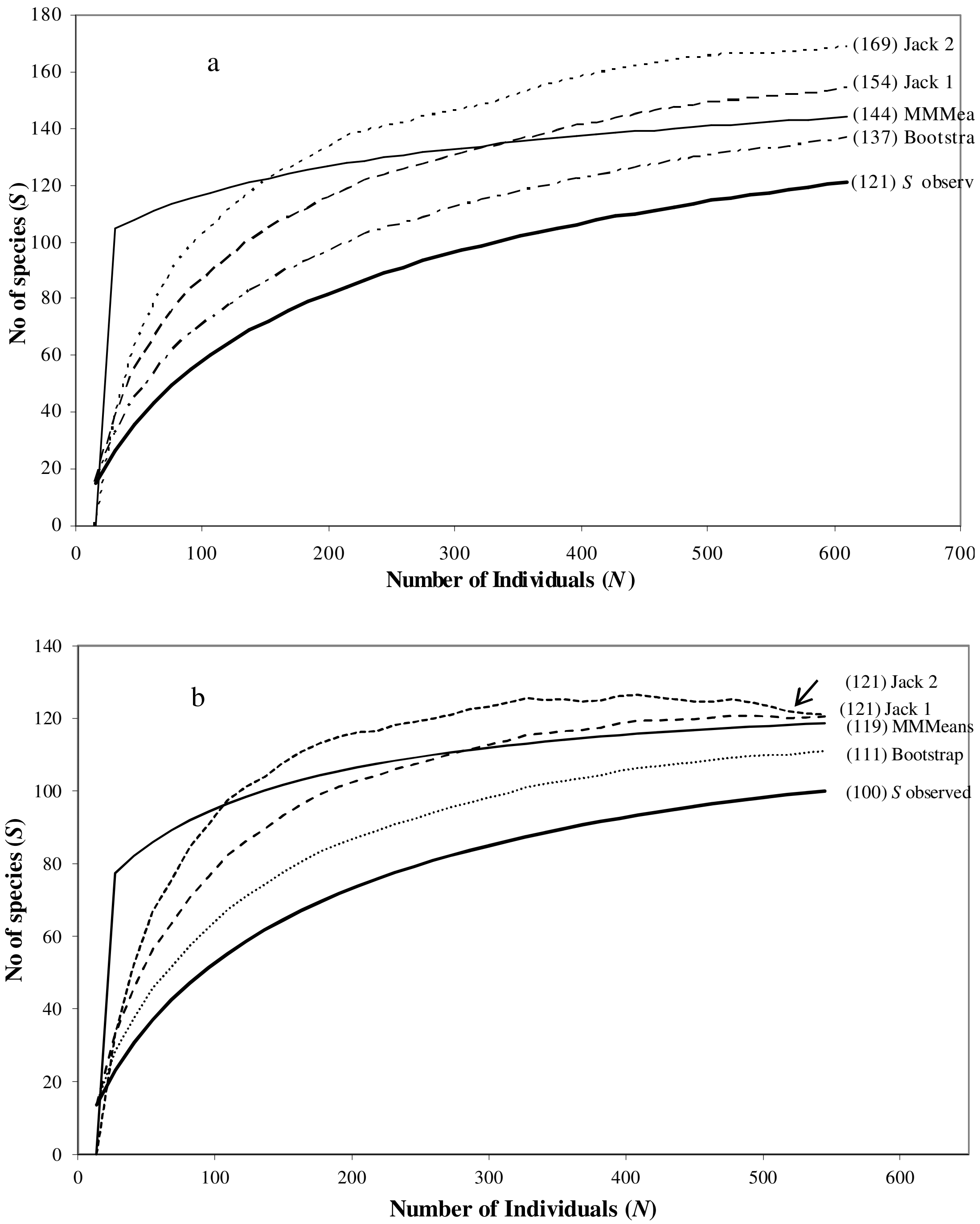

Figure 5. The performance of the non-parametric species richness estimators compared with the species accumulation curve (*S* observed) for the serpentine outcrop Groenvaly (a) and the adjacent non serpentine areas (b). The overall value of S for the randomly pooled samples is labelled at the end of each curve (in brackets).

exhibited by Figure 3, which suggest that six of the seven serpentine sites (excluding the Corezone site) have higher relative abundance than the adjacent non-serpentine areas.

The evidence (Table 4) shows that the serpentine outcrops with high species diversity also support a higher number of singletons. This supports the observation that assemblages with high species richness tend to have low evenness (Weiher and Keddy, 1999). These results suggest that the evenness index (J') selected is not sensitive enough to differentiate between the different assemblages sampled.

Table 4. Richness estimators, diversity indices and evenness values calculated for the vegetation of each serpentine outcrop and its adjacent non-serpentine vegetation.

Parameter	Core Zone	Groen-valy	Kalk-kloof	Magne-site	Mundt's Concession	Rosen-tuin	Saw-mill
Serpentine outcrops							
No. of samples (*n*)	40	40	40	40	40	40	40
No. of individuals (*N*)	396	610	642	449	446	474	492
Observed species richness (*S*)	83	121	123	105	98	115	106
Species Richness estimators							
Jack 1	109.3	154.2	154.2	143.0	123.4	156.0	143.1
Jack 2	115.5	168.9	169.8	168.0	126.8	178.3	161.6
Bootstrap	96.1	136.6	137.5	121.5	111.1	133.4	122.8
Species diversity indices							
Simpson's (-ln λ)	3.4	4.1	4.3	4.0	3.8	4.0	3.9
Shannon (H')	3.8	4.4	4.4	4.2	4.1	4.3	4.1
Fisher's α	32.0	45.3	45.2	43.1	38.8	48.3	41.5
Evenness (J')	0.86	0.91	0.92	0.90	0.90	0.90	0.89
No. of singletons	27	34	32	39	26	42	38
Adjacent non-serpentine areas							
No. of samples (*n*)	40	40	40	40	40	40	40
No. of individuals (*N*)	508	545	535	290	352	386	475
Observed species richness (*S*)	127	100	132	88	95	104	99
Species richness estimators							
Jack 1	175.8	120.5	173.9	122.1	128.2	143.0	133.1
Jack 2	207.5	121.0	187.9	141.5	143.8	163.4	149.7
Bootstrap	148.0	111.0	152.2	103.4	110.3	121.6	114.5
Species diversity indices							
Simpson's (-ln λ)	4.15	3.84	4.17	3.78	3.97	3.84	3.81
Shannon (H')	4.40	4.14	4.43	4.06	4.15	4.16	4.07
Fisher's α	54.4	35.9	56.0	43.0	42.7	46.7	38.1
Evenness (J')	**0.91**	**0.90**	**0.91**	**0.91**	**0.91**	**0.90**	**0.89**
No. of singletons	**50**	**21**	**43**	**35**	**34**	**40**	**35**

Correlation of species richness with selected environmental factors

Multiple regression analyses suggest that there is no correlation (P>0.05) between species richness and each of the selected environmental factors that is, size of outcrop, latitude, distance to nearest outcrop and levels of chromium, nickel and the calcium to magnesium ratio for each site. The same lack of correlation with selected environmental factors was shown for species diversity estimated using the Shannon, Simpson's and Fisher's indices. There is a low correlation (P=0.05) between the serpentine diversity and altitude. As altitude increase so serpentine diversity increases, but as altitude increases the non-serpentine diversity decreases. The result that on the Barberton Greenstone Belt, the serpentine species diversity is not related distance to nearest outcrop is in contrast to the vegetation of the Tuscan serpentine outcrops where evidence suggested that taxonomic diversity decreases with increasing isolation of an outcrop and decreasing surface area (Selvi, 2007). For the Californian serpentine (Harrison et al., 2006) determined that regional richness is a strong predictor of local richness, demonstrating that the regional availability of species is one key determinant of local richness. The vegetation of the eastern escarpment of the Mpumulanga Province, within which the serpentine outcrops occur, is known to support high species richness (Thuiller et al.,

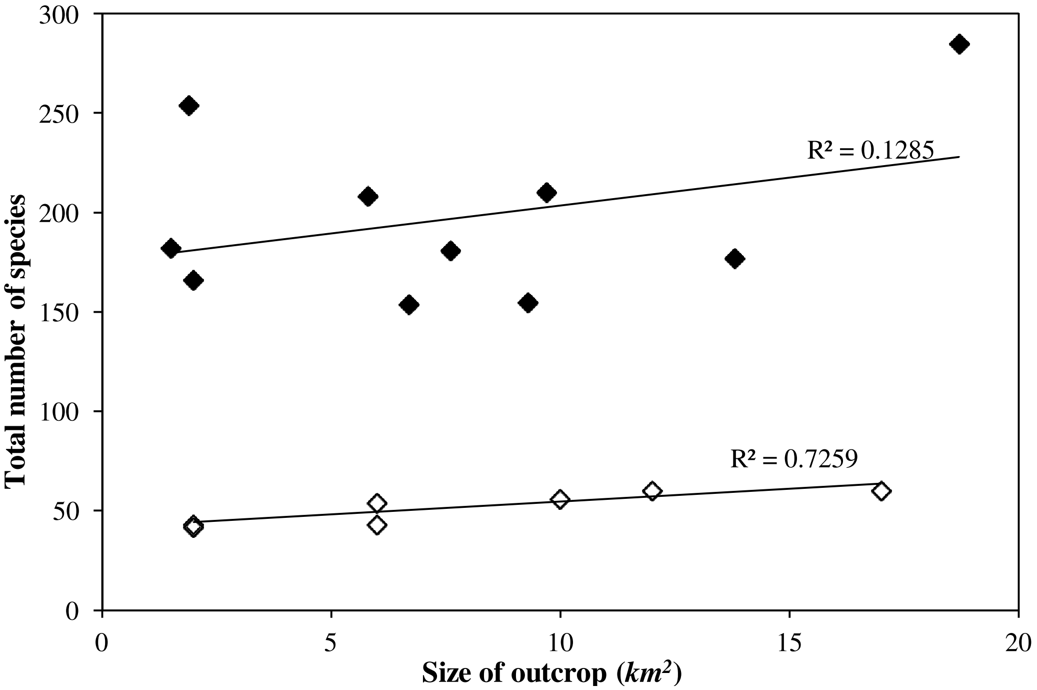

Figure 6. Scatter plot of the total number of species collected at a number of serpentine outcrops of the Barberton Greenstone Belt (♦) as compared to the number of plant species on similar sized outcrops from Tuscany (◊) in Italy (Selvi, 2007). R^2 values represent the goodness-of-fit for a linear regression calculated for each set of values. The Dunbar and Groenvaly sites are outliers with higher number of species than predicted by the line of best fit.

2006); thus it is predicted that this regional richness determines the richness of the serpentine vegetation. This is suggested by the small differences in serpentine and non-serpentine species richness and diversity. Further investigation is needed to establish the true determinants of species richness of the Barberton Greenstone Belt serpentine outcrops.

Comparisons of serpentine species richness across the world

There is huge variation in sampling strategy in the few reports of species richness of serpentine areas around the world. Werger et al. (1978) used plots of various sizes to determine the floristic diversity of the Great Dyke in Zimbabwe. Few studies of serpentine outcrops provide species richness values for individual outcrops. These factors make comparisons to the data presented here difficult. A direct comparison between the numbers of species recorded per outcrop of the Barberton Greenstone Belt and serpentine outcrops of similar size in Tuscany, Italy (Figure 6), show that the serpentines of the Barberton Greenstone Belt support many more species per outcrop than the serpentine in Tuscany (Selvi, 2007). A mean of 76 (range 26 to 205) species per serpentine was recorded for the outcrop of the Northern Apennines, Italy (Ferrari et al., 1992) which is considerably lower than the mean of 197 (range 155 to 285) species per outcrop recorded for the Barberton Greenstone Belt. The serpentine vegetation of Western North Carolina supports 27.9 species per 0.001 ha (Mansberg and Wentworth, 1984), which is higher than the serpentine of the Barberton Greenstone Belt with a mean of 23.9 species per 0.001 ha (Figure 7). The serpentine forest vegetation of the Wenatchee Mountains, Washington support 19.8 to 32 species per 0.1 ha (Del Moral, 1972), considerably lower than that of the Barberton Greenstone Belt (Figure 7).

The diversity of serpentine and non-serpentine vegetation on other continents has been compared by other authors, who have found varying patterns. The species diversity in the Siskiyou mountains is greater on serpentine than on quartz diorite or olivine gabbro except on xeric sites (Whittaker, 1960). Serpentine plant diversity (using Brillouin Index) was greater than non-serpentine diversity on xeric sites of the Wenatchee Mountains in Washington at higher elevations but not in very mesic conditions (Del Moral, 1972). On the Great Dyke in Zimbabwe, the species diversity on serpentine was found to be considerably lower than on granite or pyroxenite. In addition, it was found that on serpentine, species diversity and richness decreases with altitude (Werger et al., 1978).

In an effort to determine whether the vegetation of the serpentine outcrops has conservation value in terms of

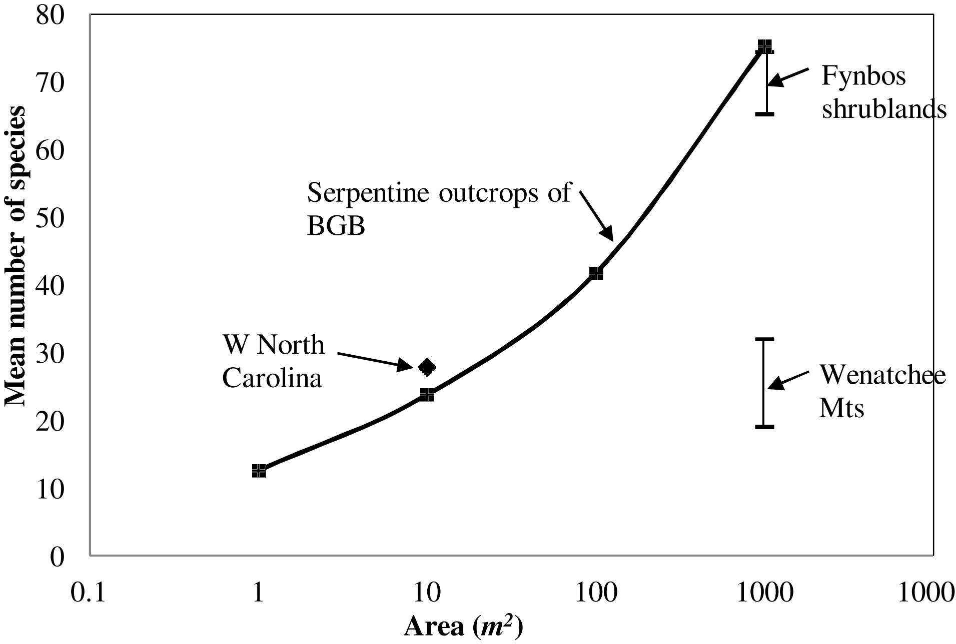

Figure 7. The mean number of species recorded in modified Whittaker plots (consisting of 1 m^2, 10 m^2, 100 m^2 and 1000 m^2 plots) for the serpentine outcrops of the Barberton Greenstone Belt (BGB) compared to the number of species recorded in the Cape Fynbos shrublands (Cowling, 1983), the serpentines of Western North Carolina (Mansberg and Wentworth, 1984) and the Wenatchee Mountains (Del Moral, 1972).

species richness, the number of species recorded per 0.1 ha plot on the serpentine of the Barberton Greenstone Belt was compared to those of the Fynbos shrubland vegetation of Cape Floral Kingdom as the Fynbos vegetation is considered to be extremely species-rich (Goldblatt, 1978). The number of species per 0.1 ha of the serpentine varies from 65.3 to 83.3 with a mean of 74.4, which is higher than the mean recorded for the Cape fynbos shrublands (66.4) (Cowling, 1983) (Figure 7). Although the two areas support decidedly different types of vegetation, the high species richness for the serpentine vegetation is an indication of potential conservation value.

Conclusion

This study represents the first attempt to comprehensively quantify the differences between the vegetation of serpentine outcrops and that of adjacent non-serpentine areas of the Barberton Greenstone Belt. We predicted that the serpentine outcrops of Barberton Greenstone Belt have been exposed sufficiently long to re-establish diversity following the geological changes associated with exposure. This study shows that the vegetation of the serpentine is not generally more species rich or diverse than the surrounding non-serpentine vegetation. However, one site showed significantly higher and a few sites slightly higher serpentine diversity, and it is possible that with more intensive sampling the species accumulation curves could show these differences to be significant. The species richness and diversity on the serpentine sites is not significantly lower than that of the surrounding vegetation, which supports Brown (1988)'s theory that the serpentine outcrops of the Barberton Greenstone Belt were exposed sufficiently long ago to allow the vegetation to re-establish diversity after the initial geological disturbance. The Core Zone site has significantly lower species richness and diversity, and it is possible that the isolation of this outcrop has resulted in its lower diversity.

The flora of the Barberton Greenstone Belt seems to be distinct from the surrounding vegetation. Quantifying this distinction, the Sørenson's index, comparing the species composition on and off serpentine for each site, suggests that although species richness is similar on and off serpentine, there is a considerable change in species composition across the serpentine to non-serpentine 'boundary'. This supports the view that serpentine outcrops support a unique and diverse flora. Further research is required to determine whether grassland species in this area are less able to colonise serpentine soils than bushveld species, as suggested by the correlation in Table 2. A further hypothesis that should be tested in the future is that sites that are geographically clustered are more similar to each other than to their adjacent non-serpentine vegetation.

Identification of the factors affecting diversity is essential for determining conservation priorities for these serpentine outcrops. There is considerable variation in the species richness and diversity calculated for each site sampled, with the sites in the southern parts of the Barberton Greenstone Belt having higher richness than the northern sites. However, the differences could not be correlated to latitude or any other climatic or edaphic factor. Further investigations, sampling the vegetation of more outcrops and considering additional environmental factors should reveal the factors determining diversity on the various outcrops and the extent of the altitudinal correlation with species diversity.

The serpentine sites of the Barberton Greenstone Belt show considerably higher species richness than that reported for the serpentines of Tuscany (Ferrari et al., 1992) and the serpentine forest vegetation of the Wenatchee Mountains, Washington (Del Moral, 1972) and slightly lower species richness than the serpentine vegetation of Western North Carolina (Mansberg and Wentworth, 1984). Further comparisons could not be made as additional comparable data could not be found and, therefore, no general statements can be made as to how the vegetation of the serpentine of the Barberton Greenstone Belt compares to other sites around the world in terms of species diversity.

The species richness and diversity values calculated here, together with levels of endemism still to be published, will form part of a larger study which will state a case for including serpentine vegetation in the planning for conservation of the vegetation of the eastern portion of Mpumalanga Province.

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