

A preliminary survey of the diversity of soil algae and cyanoprokaryotes on mafic and ultramafic substrates in South Africa

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Abstract. Despite a large body of work on the serpentine-substrate effect on vascular plants, little work has been undertaken to describe algal communities found on serpentine soils derived from peridotite and other ultramafic rocks. We report a preliminary study describing the occurrence of algae and cyanoprokaryotes on mafic and ultramafic substrates from South Africa. Results suggest that slope and aspect play a key role in species diversity and community composition and, although low pH, nutrients and metal content do not reduce species richness, these edaphic features also influence species composition. Further, typical soil genera such as *Leptolyngbya*, *Microcoleus*, *Phormidium*, *Chlamydomonas*, *Chlorococcum* and *Hantzschia* were found at most sites. *Chroococcus* sp., *Scytonema ocellatum*, *Nostoc linckia*, *Chlorotetraedron* sp., *Hormotilopsis gelatinosa*, *Klebsormidium flaccidum*, *Pleurococcus* sp. and *Tetracystis elliptica* were unique to one serpentine site. The preliminary survey provides directions for future research on the serpentine-substrate effect on algal and cyanoprokaryote diversity in South Africa.

Additional keywords: algae, cryptogamic ecology, serpentine geocology, species diversity.

Introduction

A range of soils can develop from ultramafic rocks depending on climate, time, relief, chemical composition of the parent materials as well as biotic factors, especially plants and microbes (Proctor and Woodell 1975; Cardace *et al.* 2014). Serpentine soil is derived from serpentinite, a rock formed primarily by the hydration and metamorphic transformation of the ultramafic rock, peridotite. Ultramafic rocks can vary greatly in chemical and mineral composition and can be composed of various combinations of the minerals olivine, orthorhombic and monoclinic pyroxenes, hornblende as well as the secondary products of these minerals such as serpentine group minerals, including fibrous amphiboles and talc (Alexander *et al.* 2007). Serpentine soils have elevated levels of heavy metals such as nickel (Ni) and chromium (Cr), near-neutral to alkaline pH values, and calcium:magnesium (Ca:Mg) ratios <1 (Rajakaruna *et al.* 2009). Serpentine soils are also generally characterised by nutrient deficiencies, especially nitrogen (N), phosphorus (P) and potassium (K) (Daghino *et al.* 2012). The distinctive chemistry of ultramafic rocks and resulting serpentine soils restricts the growth of many plants, making such sites refuges for those plants that can thrive under the serpentine influence (Alexander *et al.* 2007). Serpentine habitats are known to harbour high numbers of endemic plant

species (Siebert *et al.* 2002; O'Dell and Rajakaruna 2011) and are model settings for the study of plant ecology and evolution (Harrison and Rajakaruna 2011). However, cryptogamic species (i.e. such as lichens, bryophytes, algae, cyanoprokaryotes) show low levels of edaphic endemism (Bramwell and Caujape-Castells 2011; Rajakaruna *et al.* 2012) and appear to be broadly tolerant of substrate, resulting in wide geographic distributions and range disjunctions that frequently span more than one continent (Schuster 1983). Species with wide distributions sometimes undergo environmental modification and exhibit habitat-associated vagrant forms (i.e. morphotypes). For example, Rosentreter and McCune (1992) documented how soil, biota and climate can interact to produce partially or completely vagrant life forms in several lichen genera.

According to Belnap and Lange (2001), algae and cyanoprokaryotes can colonise almost all soil types. Terlizzi and Karlander (1979) found members of the Cyanophyceae, Chlorophyceae and Bacillariophyceae in serpentine soil samples collected at Soldiers Delight, Maryland, USA, but concluded that the composition of the soil flora at the division level is similar to that of more favourable soil types. Serpentine soil environments are comparable to metal-enriched mine tailings that include stressors such as nutrient deficiencies, unfavourable

soil structure, water stress and toxic concentrations of metals (Reddy *et al.* 2001; O'Dell and Rajakaruna 2011). Orlekowsky *et al.* (2013) found that algae and cyanoprokaryotes are able to colonise mine tailings despite the harsh conditions and speculated that the presence of higher plants might have provided a microenvironment for the establishment of these organisms. Rosentreter and McCune (1992) found that vascular plants create windbreaks and shade, influencing moisture content and light intensity at the soil surface and creating suitable habitats for microbes. Cabala *et al.* (2011) concluded that crust formation in soils with a low pH and heavy-metal contamination is possible, but an increase in moisture and pH promotes algal development. According to Lukesova (2001), Bacillariophyceae and Cyanophyceae are more characteristic of alkaline environments, whereas species of Chlorophyceae can colonise more acidic soils.

Moisture is a key factor in the establishment of algae and cyanoprokaryotes. The low plant cover often associated with exposed and rocky serpentine outcrops contributes to hot and dry soil surfaces (Kruckeberg 2002), making it uninhabitable for soil-dwelling algae and cyanoprokaryotes. Surface temperature is also important because it regulates many ecosystem functions such as rates of N and carbon fixation, soil water evaporation and microbial activity (Belnap 2003). Kruckeberg (2002) proposed that the microbial biota of serpentine soil would be species poor, paralleling the scanty vegetation, but admitted that very little is known about the microbes of serpentine soils.

Since Kruckeberg (2002), numerous studies have documented the diversity of bacteria (Oline 2006; Rajkumar *et al.* 2008), fungi (DeGroot *et al.* 2005; Daghino *et al.* 2012; Southworth *et al.* 2014) and lichens (Favero-Longo *et al.* 2004; Rajakaruna *et al.* 2012) in serpentine soil, yet investigations of algae and cyanoprokaryotes in ultramafic soil (Terlizzi and Karlander 1979; Couté *et al.* 1999; Hauer 2008) are still scarce. The aim of the present study was to conduct a preliminary survey of the algal and cyanoprokaryote composition in mafic- and ultramafic-derived soil to improve our understanding of the microbial diversity of serpentine and related soils.

Materials and methods

Sampling sites

Soil was sampled in February 2012 at different sampling localities in Mpumalanga and Limpopo provinces of South Africa (Fig. 1, Table 1). Ohrigstad sites (Sites 1 and 2, which were siliciclastic rocks and Silverton Formation) were situated on the metasediments of the Paleoproterozoic Transvaal Supergroup that forms the floor to the mafic-ultramafic Rustenburg Layered Suite of the Bushveld Igneous Complex (Clarke *et al.* 2009). The Bushveld Complex was formed 2000 million years ago when enormous volumes of magma intruded the upper levels of the earth crust (Clarke *et al.* 2009). Steelpoort sites (Sites 3 and 4) were situated on the ultramafic pyroxenite hills of the Vlakfontein Subsuite of the Rustenburg Layered Suite and the Burgersfort sites (Sites 5 and 6) on mafic hills of Kolobeng norite. The vegetation of Sites 1–6 (Table 1) had an open to dense woody layer, including woody and herbaceous shrubs, and an open to closed grass layer (Mucina and Rutherford 2006). It was found on moderate to steep mountainsides.

Sites 7 and 8 were located on amphibolites and serpentinite of the lower-most greenstone formations of the Barberton Supergroup (Norman and Whitfield 2006). These lower formations (Sites 7 and 8) are metamorphic and made up of the volcano-sedimentary Onverwacht Group that consists of ultramafic rocks that formed 3500 million years ago and minor felsic volcanic and sedimentary rocks that were deposited in an ancient marine environment (Hofmann *et al.* 2012). The vegetation of Sites 7 and 8 is considered herbaceous savanna with an open woody layer and dense forb and grass layer (Mucina and Rutherford 2006). It is generally associated with hilly terrain, with steep to moderate slopes.

Mean annual temperatures of the study area vary between 16.7 C and 18 C and a mean annual summer rainfall ranges from 518 to 1194 mm (Mucina and Rutherford 2006; see Table 1).

At each site, a disposable plastic teaspoon was used to scrape soil up to a depth of 3 mm at three localities not more than 50 m apart, namely, underneath low-growing vegetation (mostly grasses), in bare soil and between rocks. A composite sample, comprising nine subsamples (teaspoons), was made for each of the eight sites and mixed thoroughly.

Identification

A combination of direct-determination and enrichment-culture techniques were used to detect cyanoprokaryotes and eukaryotic algae (Langhans *et al.* 2009). A direct investigation was conducted according to the method described in Lund (1945). Subsamples of 10 g of soil were wetted with distilled water in Petri dishes and incubated at 20 C and a light intensity of $35 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Three sterile coverslips were placed on the soil in each Petri dish after 24 h. Algal communities that established underneath the coverslips were examined and enumerated using the semiquantitative scale of Starmach (1963), which classifies algae and cyanoprokaryotes as subdominant if 30–50 specimens are present on the coverslip and dominant if more than 50 specimens are present.

The enrichment-culture techniques included the use of agar plates and liquid cultures. For the agar plates, 10-g soil subsamples were incubated on 1.5% agar plates enriched with either Bold's basal growth medium (BBM; Brown *et al.* 1964) as described in Stein (1973) or GBG11 growth medium (Krüger 1978), and incubated at 20 C and a light intensity of $35 \mu\text{mol m}^{-2} \text{ s}^{-1}$. For the liquid cultures, subsamples of 10 g of soil each were enriched with either 100 mL BBM (Stein 1973) or 100 mL GBG11 growth medium (Krüger 1978) and incubated at a temperature of 20 C and a continuous light intensity of $15 \mu\text{mol m}^{-2} \text{ s}^{-1}$ to stimulate the growth of algae and cyanoprokaryotes present in low concentrations. Two different growth media were used because green algal growth is enhanced by BBM (Stein 1973) and cyanoprokaryotes by GBG11 growth medium (Krüger 1978). Cyanoprokaryotes also prefer lower light intensities and this was implemented during the incubation of the cultures.

The species were identified microscopically using a Nikon 80i microscope with differential interference contrast, 'n 60X Plan Apochromatic 1.4 numerical aperture (NA) oil violet-corrected with a 1.4 NA oil condenser. Literature used for identification included Ettl *et al.* (1999), Hindak (2008),

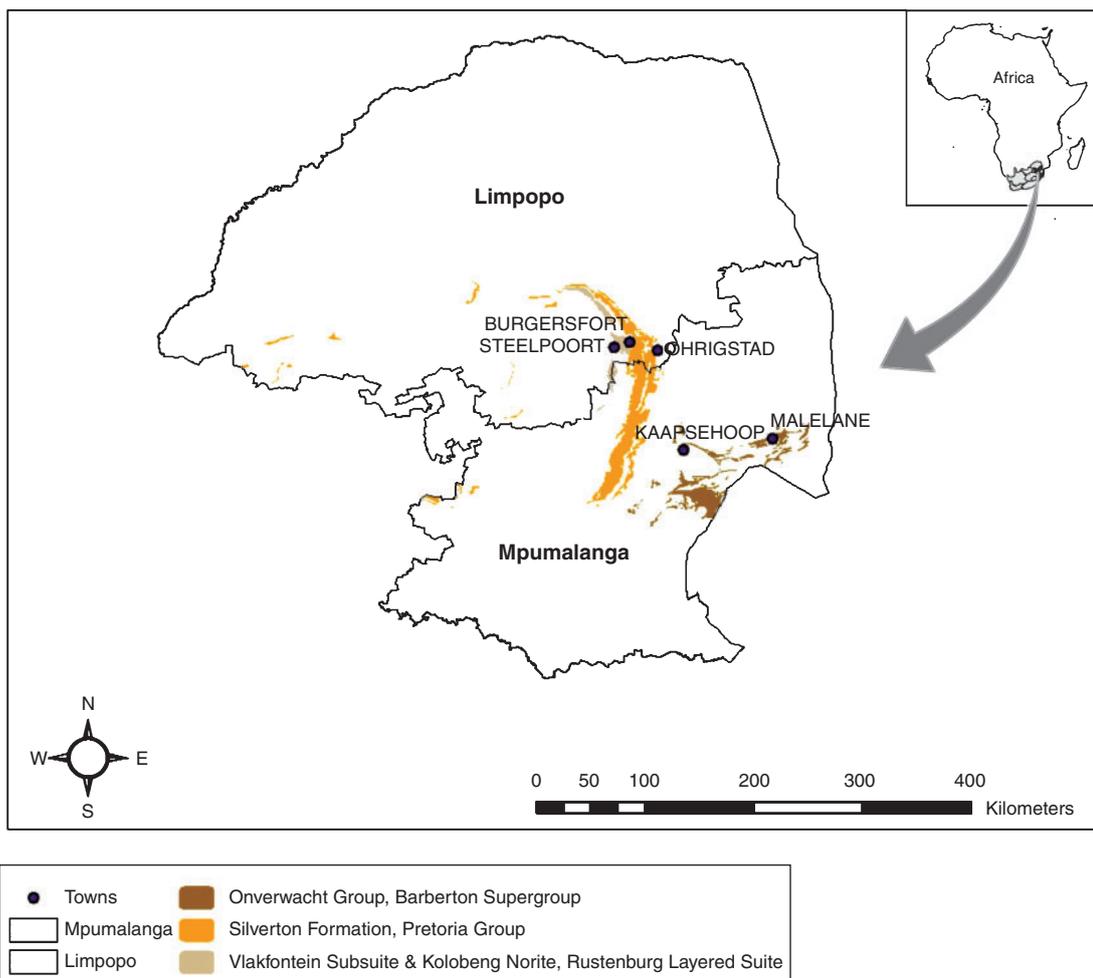


Fig. 1. Geology and locations of towns nearest to the sampling localities. Sites 1, 2 are near Ohrigstad, Sites 3, 4 are near Steelpoort, Sites 5, 6 are near Burgersfort, Site 7 is near Malelane, and Site 8 is near Kaapsehoop. Sites 7 and 8 are serpentinite-derived.

Hüber-Pestalozzi (1961), John *et al.* (2002), Komárek and Anagnostidis (2005), Taylor *et al.* (2007) and Wehr and Sheath (2003).

Soil analyses

At each site, the soil was randomly sampled from five of the nine subsamples per locality (minimum one and maximum two per site) and pooled to make a composite sample. The soil analysis was performed in accordance with the standards set out by the control schemes of the Agricultural Laboratory Association of Southern Africa and the International Soil Analytical Exchange (ISE), Wageningen, The Netherlands. Exchangeable Ca, Mg, K and sodium (Na) were estimated by 1M ammonium acetate (pH=7), P was estimated by P-Bray 1 extraction, pH was estimated via 1:2.5 extraction and electrical conductivity (EC) was determined with a saturated extraction. These methods followed NSSSA (1990). All heavy metals, including aluminium (Al), arsenic (As), cadmium (Cd), cobalt (Co), copper (Cu), Cr, iron (Fe), mercury (Hg), manganese (Mn), molybdenum (Mo), Ni, lead (Pb) and vanadium (V), as well as %N and %S were estimated by EPA Method 3050b (US EPA 1996).

Data analysis

Statistica version 12 software (StatSoft Inc., Tulda, OK, USA) was used to perform Student's *t*-tests to determine whether the data from one site differed significantly ($P < 0.5$) from those from another site. The algal composition of a site was compared with the algal composition of another site and repeated until each site was compared with all the other sites. The approach was also repeated for the edaphic features of each site. Similarities among the species compositions of the different sites were analysed by using the Bray–Curtis dissimilarity index (Hahs and McDonnell 2006). This was performed with the software program Primer 5 (Clarke and Gorley 2001). CANOCO version 4.5 software (Cambridge University Press, Cambridge, UK) was used to perform principal component analysis (PCA) on the chemical variables of the different sites. Canonical correspondence analysis (CCA) was performed on the species data as well as the chemical variables (Table 2), which included the Monte Carlo permutation test for significance (Ter Braak and Smilauer 1998). The species matrix for the analysis was compiled by allocating values to the species that were absent (0), present (1) subdominant (2) and dominant (3).

Table 1. List of algae and soil sampling localities in Mpumalanga and Limpopo, South Africa, including selected environmental variables and descriptions

Site number	Nearest town	Strata name	Lithic class	Aspect	Vegetation unit	Mean annual rainfall (mm)	Mean annual temperature (C)	Coordinates
1	Ohrigstad	Silverton Formation, Pretoria Group	Siliciclastic rocks	Foot slope, northern aspect	Ohrigstad Mountain Bushveld	645	18.0	24 45.586'S, 30 23.307'E
2	Ohrigstad	Silverton Formation, Pretoria Group	Siliciclastic rocks	Upper slope, northern aspect	Ohrigstad Mountain Bushveld	645	18.0	24 45.535'S, 30 23.304'E
3	Steelpoort	Vlakfontein Subsuite, Rustenburg Layered Suite	Ultramafic rocks	Foot slope, western aspect	Sekhukhune Mountain Bushveld	609	17.5	24 29.673'S, 30 07.993'E
4	Steelpoort	Vlakfontein Subsuite, Rustenburg Layered Suite	Ultramafic rocks	Upper slope, western aspect	Sekhukhune Mountain Bushveld	609	17.5	24 29.618'S, 30 08.020'E
5	Burgersfort	Vlakfontein Subsuite, Rustenburg Layered Suite	Mafic rocks	Upper slope, north-western aspect	Sekhukhune Plains Bushveld	518	19.0	24 40.364'S, 30 16.743'E
6	Burgersfort	Vlakfontein Subsuite, Rustenburg Layered Suite	Mafic rocks	Foot slope, north-western aspect	Sekhukhune Plains Bushveld	518	19.0	24 40.369'S, 30 16.731'E
7	Malelane	Onverwacht Group Baberton Supergroup	Metamorphic rocks	Foot slope, northern aspect	Barberton Serpentine Sourveld	884	18.4	25 31.997'S, 31 31.050'E
8	Kaapschehoop	Onverwacht Group Baberton Supergroup	Metamorphic rocks	Rocky ridge, north-western aspect	Barberton Serpentine Sourveld	1194	16.7	25 32.892'S, 30 48.962'E

Table 2. Chemical characteristics for composite soil samples collected from the eight sites
EC, electrical conductivity; CEC, cation exchange capacity

Soil characteristic	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
pH (H ₂ O)	6.27	5.54	6.46	6.36	6.86	6.31	7.61	5.91
pH (KCl)	5.31	4.81	5.21	5.49	6.15	5.38	6.8	5.34
EC (mS m ⁻¹)	23	31	28	31	58	39	55	10
CEC (cmol(+) kg ⁻¹)	11.44	13.49	24.68	25.20	25.95	25.18	18.39	3.76
%Base saturation	66.25	48.01	54.36	67.84	93.17	72.14	109.4	46.9
Macronutrients								
Calcium (Ca) (cmol(+) kg ⁻¹)	5.07	3.49	5.82	6.17	14.61	8.4	8.22	0.36
Magnesium (Mg) (cmol(+) kg ⁻¹)	1.98	2.23	11.25	10.65	9.06	9.18	11.51	1.36
Ca : Mg ratio	2.56	1.57	0.52	0.58	1.61	0.92	0.71	0.26
Potassium (K) (mg kg ⁻¹)	195.5	281	102.9	95	188.5	218	136.5	1.5
Sodium (Na) (mg kg ⁻¹)	6	8	7	7	6	7	9	9
Phosphorus (P) (mg kg ⁻¹)	3.6	4.3	21.2	10.8	3.9	37.7	4.2	1.7
%Nitrogen	0.46	0.44	0.52	0.49	0.58	0.63	0.47	0.4
%Sulfur	0.21	0	0.01	0.01	0	0.04	0	0.01
Micronutrients and heavy metals (mg kg⁻¹)								
Aluminium	2746	3514	826	921	1959	1514	1555	3021
Arsenic	0.08	0.1	0.1	0.08	0.07	0.15	0.43	4.06
Cadmium	0.001	0.001	0.01	0.001	0.0008	0.003	0.0008	0.0009
Cobalt	1.38	1.79	3.48	3.92	2.2	4.79	3.78	15.14
Copper	1.17	1.53	0.85	0.6	1.74	1.26	4.35	8.31
Chromium	6.26	7.94	85.29	93.97	32.45	124.4	55.42	134.2
Iron	3098	3733	3832	3377	2271	4701	4274	8814
Mercury	0.0004	0.0007	0.0046	0.0005	0.0002	0.0004	0.0006	0.0004
Manganese	47.33	60.21	60.35	78.73	51.68	108.8	93.6	167
Molybdenum	0.02	0.03	0.03	0.02	0.02	0.04	0.02	0.04
Nickel	4.02	5.03	36.25	29.88	17.48	31.27	33.86	48.33
Palladium	0.01	0.02	0.01	0.01	0.01	0.02	0.02	0.03
Vanadium	8.51	10.11	4.24	3.39	4.63	5.21	7.96	20.62

Results

Soil analyses

All soils were slightly acidic, except for serpentine Site 7 where a pH (H₂O) of 7.61 was reported (Table 2). The highest concentrations of K and Al were measured at the upper slope of the siliciclastic soils at Ohrigstad (Site 2) and the highest concentrations of Cd and Hg were measured at the foot-slope site near Steelpoort (Site 3). Burgersfort mafic soils had the highest concentrations of Ca (Site 5) and P (Site 6).

The first two axes of the PCA of the soil characteristics and the sampling sites explained 71.6% of the total variance (Fig. 2). It showed a clear association of heavy metals such as As, Co, Cu, Cr, Fe, Mn, Mo, Ni, Pd and V with the serpentine site at Kaapschehoop (Site 8) that also features other characteristics of serpentine soils such as low concentrations of essential nutrients, including a Ca:Mg ratio of <1. This was substantially lower than for other ultramafic and mafic rocks (Sites 3, 4, 6) with ratios <1. All mafic and ultramafic sites had heavy-metal (mostly Ni) concentrations exceeding those of the sedimentary rocks and comparable to concentrations found at the two serpentine sites.

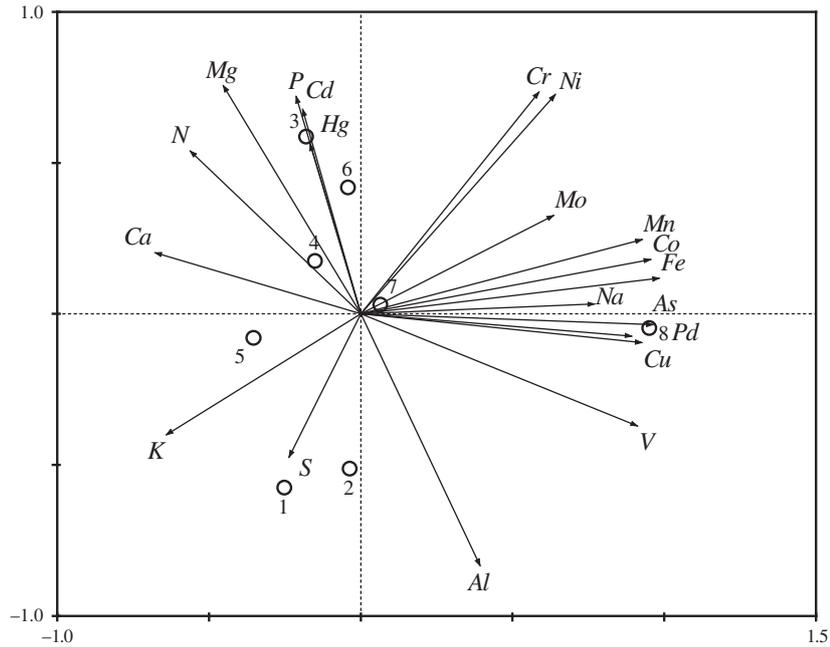
The cation exchange capacity (CEC) of the serpentine soil at Site 8 was the lowest among the sites (3.76 cmol(+) kg⁻¹). This is below the minimum standard of 8 cmol(+) kg⁻¹ proposed for agricultural top soil in South Africa by the Soil Classification Working Group (1991). The EC of Site 8 soil was low compared

to the other sites, likely resulting from low K concentrations present at this site.

A Student's *t*-test indicated that the edaphic features (see Table 2 for soil features tested) of the siliciclastic Site 2 and the serpentine Site 8 differ significantly ($P=0.001$). Sites 1, 2 and 8 had the highest concentrations of Al, a known soil toxin in South Africa, but the pH of Site 2 and Site 8 was lower (5.54 and 5.91, respectively) than the pH of Site 1 (6.27), which could play a role in the bioavailability of heavy metals. For instance, at an acidic pH, Al exists only in one oxidation state (+3) and can react with other matter in the environment to form various complexes (ATSDR 2008).

Algal diversity

Site 1 had the highest number of species (24), followed by serpentine Sites 7 (22) and 8 (20) (Table 3). The lowest number of species (6) was found on the pyroxenite-derived soil at Site 3. Cyanophyceae diversity was highest at Sites 1, 4, 7 and 8 and dominated at all sites except at Site 3 where *Hantzschia amphioxys* (Bacillariophyceae) was the dominant algae. *Leptolyngbya* sp. was dominant at Sites 1, 4, 5, 7 and 8 and subdominant at Site 6. *Phormidium* was dominant at Sites 2, 4, 5 and 7 and subdominant at Sites 3, 6 and 8. *Microcoleus* sp. was subdominant in the siliciclastic sites near Burgersfort (Sites 1, 2) as well as the serpentine sites (Sites 7 and 8), and dominant at the mafic site near Burgersfort (Site 6). *Leptolyngbya foveolarum*,



Legend: ○ sites; → environmental variables

Axis	1	2	3	4
Eigen values	0.48	0.235	0.093	0.091
Cumulative percentage variance:				
Of species	48	71.6	80.8	90

Fig. 2. Principal component analysis (PCA) to show the relationship between soil characteristics and the sampling sites.

Phormidium ambiguum and *Hantzschia amphioxys* were found at all sites. Chlorophyceae was the most diverse group, with the highest diversity at Site 1, whereas Bacillariophyceae diversity was the highest at Sites 1, 4 and 5. The Cyanophyceae, Chlorophyceae and Bacillariophyceae were represented at every site. However, Eustigmatophyceae did not occur at Sites 3 and 4 and the Xanthophyceae occurred only at Sites 2, 6 and 7.

Preliminary correlations between edaphic features and algal diversity

Multivariate analysis confirms the strong association of heavy metals with the serpentine soils at Site 8 (Figs 2, 3). The first two axes of the CCA (Fig. 3) explained only 36.2% of the variance. Variables with high inflation factors (Ca, Mg, P and N) were removed to improve the analysis, but eigenvalues or the explanation of the total variance did not change. The forward selection of factors lowered the *P*-value; however, it was still not significant, so we kept all the variables. Even with the low values, the CCA helps to visualise the species distribution with respect to the different sites, and presents preliminary information on how different edaphic features are correlated with algal diversity. *Hormotilopsis gelatinosa*, *Klebsormidium flaccidum*, *Pleurococcus* species, *Chlorotetraedron* species and *Tetracystis elliptica* belonging to the Chlorophyceae, and *Chroococcus*

species, *Scytonema ocellatum*, *Nostoc linckia* as well as an unknown *Nostoc* species from the Cyanophyceae were closely associated with Site 8 (Fig. 3). High concentrations of K were associated with the upper slope of the sedimentary soils in Ohrgstad (Site 2; Figs 2, 3) as well as the green algae *Characiopsis minima*, *Chlamydomonas macrostellata*, *Bumilleriopsis filiformis* from the Xanthophyceae and the cyanoprokaryote *Oscillatoria raoui*. Calcium, Mg, S and N appear to play a role in the similarity in species composition of Sites 1, 3, 4 and 5.

Bray–Curtis dissimilarity index (Hahs and McDonnell 2006) grouped samples from lower slopes (Sites 1, 6 and 7; indicated with black square in Fig. 3), whereas the other sites were scattered (stress value 0.05). Cyanoprokaryotes such as *Leptolyngbya foveolarum*, *Microcoleus vaginatus* and *Phormidium ambiguum*, Chlorophyceae such as *Chlamydomonas* and *Chlorococcum* species, Eustigmatophyceae such as *Eustigmatos magnus* and *Hantzschia amphioxys* from Bacillariophyceae occurred on all the lower-slope sites (Sites 1, 6 and 7), but were not unique to these sites. The cyanoprokaryote *Phormidium ambiguum* was dominant in all the upper-slope sites (Sites 2, 4 and 5). It was interesting that the serpentine sites (Sites 7 and 8) did not form a group. Nor did the mafic and ultramafic sites (Sites 3, 4, 5 and 6) or siliciclastic sites (Sites 1 and 2). This random pattern, with a lack of grouping for sites, showed that slope position (upper vs lower) had a larger effect on

Table 3. Algae and cyanoprokaryotes identified from the different soil samples collected from the eight sites
dom, dominant; s/dom, subdominant; +, present

Species	Abbreviations used in Fig. 3	Siliciclastic rocks		Mafic and ultramafic rocks				Serpentine	
		Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
Cyanophyceae									
<i>Chroococcus</i> sp.	Chro								+
<i>Komvophoron</i> cf. <i>schmidlei</i> (Jaag)	Kom	+							
Anagnostids et Komárek									
<i>Leptolyngbya foveolarum</i> (Rabenhorst ex Gomont) Anagnostidis et Komárek	Lepto	dom	+	+	dom	dom	s/dom	dom	dom
<i>Lyngbya major</i> Meneghini	Lyn1	+		+		+			
<i>Lyngbya</i> cf. <i>truncicola</i> Ghose	Lyn2				+				
<i>Microcoleus vaginatus</i> (Vauch.) Gom.	Micro	s/dom	s/dom				dom	s/dom	s/dom
<i>Nostoc commune</i> Vauch. sensu Elenk.	Nos1			+					
<i>Nostoc linckia</i> (Roth.) Born. et Flah. in sensu Elenk.	Nos2								s/dom
<i>Nostoc punctiforme</i> (Kütz.) Hariot	Nos3							+	
<i>Nostoc</i> sp.	Nos4								+
<i>Oscillatoria limosa</i> Ag.	Osc1				+			+	
<i>Oscillatoria raoi</i> De Toni	Osc2		+						
<i>Phormidium ambiguum</i> Gomont	Pho1	+	dom	s/dom	dom	dom	s/dom	dom	s/dom
<i>Phormidium animale</i> (Agardh ex Gomont) Anagnostidis et Komárek	Pho2				+				
<i>Phormidium</i> cf. <i>corium</i> (Ag.) Kütz. ex Gomont	Pho3						+		
<i>Phormidium</i> cf. <i>jenkelianum</i> Schmid	Pho4					+			
<i>Phormidium jadinianum</i> Gomont	Pho5				+				
<i>Phormidium mucicola</i> Naum. et Hub.-Pestalozzi	Pho6						+		
<i>Pseudanabaena</i> cf. <i>minima</i> (G.S.An) Anagnostidis	Pseu1				+				
<i>Pseudanabaena frigida</i> (Fritsch) Anagnostidis	Pseu2							+	
<i>Pseudanabaena</i> sp.	Pseu3	+			+			+	
<i>Scytonema myochrous</i> (Dillw.) Ag. ex Born. et Flah.	Scyt1							+	dom
<i>Scytonema ocellatum</i> Born. et Flah.	Scyt2								dom
<i>Scytonema</i> sp.	Scyt3	+							
<i>Synechocystis crassa</i> Woronich.	Syn				+				
Total Cyanophyceae		7	4	4	9	4	5	8	8
Chlorophyceae									
<i>Bracteacoccus</i> cf. <i>grandis</i> Bischoff et Bold	Brac1	+							
<i>Bracteacoccus minor</i> (Chod.) Petrová	Brac2	+							
<i>Bracteacoccus</i> sp.	Brac3							+	
<i>Characiopsis minima</i> Pascher	Chara		+						
<i>Chlamydomonas macrostellata</i> Lund	Chlamy		s/dom						
<i>Chlamydomonas</i> sp.1	Chlam1	+	+		+		+	+	+
<i>Chlamydomonas</i> sp.2	Chlam2				+				
<i>Chlorella munitissima</i> Fott et Nováková	Chlo1	+							
<i>Chlorella vulgaris</i> Beijerinck	Chlo2	+				s/dom	+		
<i>Chlorella</i> sp.	Chlo3					+		+	
<i>Chlorococcum echinozygotum</i> Starr	Chlc1	s/dom							
<i>Chlorococcum infusionum</i> (Schränk) Meneghini	Chlc2	+			+	+	+	+	
<i>Chlorococcum oviforme</i> Archibald et Bold	Chlc3						+		
<i>Chlorococcum vacuolatum</i> Starr	Chlc4					+			
<i>Chlorococcum</i> sp.1	Chlc5	+	+						
<i>Chlorococcum</i> sp.2	Chlc6	+			+		+	+	+

(continued next page)

Table 3. (continued)

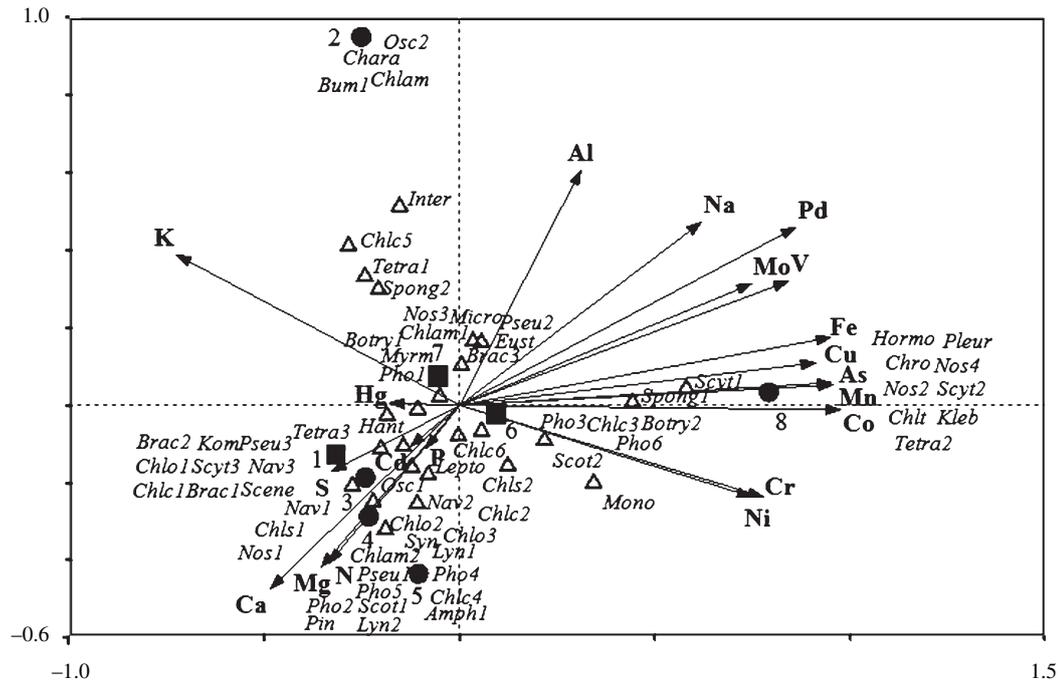
Species	Abbreviations used in Fig. 3	Siliciclastic rocks		Mafic and ultramafic rocks				Serpentine	
		Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
<i>Chlorosarcinopsis aggregata</i> Arce et Bold	Chls1			+					
<i>Chlorosarcinopsis minor</i> (Gerneck) Herndon	Chls2					s/dom	+	+	
<i>Chlorotetraedron</i> sp.	Chlt								+
<i>Hormotilopsis gelatinosa</i> Trainor et Bold	Hormo								+
<i>Interfilum</i> sp.	Inter		+					+	
<i>Klebsormidium flaccidum</i> (Kütz.) P.C.Silva, Mattox et W.H.Blackwell	Kleb								+
<i>Myrmecia biatorellae</i> (Tschermak-Woess) J.B.Petersen	Myrm							+	
<i>Pleurococcus</i>	Pleur								+
<i>Scenedesmus</i> sp.	Scene	+							
<i>Scotiellopsis rubescens</i> Vinatzer	Scot1				+				
<i>Scotiellopsis terrestris</i> (Reisigl) Punčochár ová et Kalina	Scot2				+		+		+
<i>Spongiochloris</i> sp.1	Spong1						+		+
<i>Spongiochloris</i> sp.2	Spong2	+	+					+	
<i>Tetracystis aggregata</i> Brown et Bold	Tetra1		+		+				
<i>Tetracystis elliptica</i> Nakano	Tetra2								+
<i>Tetracystis</i> sp.	Tetra3	+						+	
Total Chlorophyceae		12	7	1	7	5	8	10	9
Eustigmatophyceae									
<i>Eustigmator magnus</i> (J.B.Petersen) Hibberd	Eust	+	+				+	s/dom	+
<i>Monodopsis subterranea</i> (J.B.Petersen) Hibberd	Mono					+			+
Total Eustigmatophyceae		1	1	0	0	1	1	1	2
Xantophyceae									
<i>Botrydiopsis arhiza</i> Borzi	Botry1							s/dom	
<i>Botrydiopsis</i> sp.	Botry2						+		
<i>Bumilleriopsis filiformis</i> Vischer	Bum1		s/dom						
Total Xantophyceae		0	1	0	0	0	1	1	0
Bacillariophyceae									
<i>Amphora veneta</i> Kützling	Amph1					+			
<i>Pinnularia borealis</i> Ehrenberg	Pin	+			+	s/dom			
<i>Navicula mutica</i> Kützling	Nav1	+			+				
<i>Navicula pelliculosa</i> (Kützling) Hilse	Nav2					+		+	
<i>Navicula veneta</i> Kützling	Nav3	+							
<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow	Hant	+	s/dom	dom	s/dom	s/dom	+	+	+
Total Bacillariophyceae		4	1	1	3	4	1	2	1
Total species richness		24	14	6	19	14	16	22	20

diversity. Cyanoprokaryote species were less associated with the foot slope of pyroxenite–magnesium rich soil in Steelpoort (Site 3), with a Student's *t*-test supporting a significant difference from those at Sites 1 ($P=0.03$), 4 ($P=0.03$), 7 ($P=0.007$) and 8 ($P=0.01$). The pattern also showed that the lithic class may be less important than the slope position, in determining the algal and cyanoprokaryote species present at the sites.

Discussion

It is widely reported that cyanoprokaryotes are well adapted to a wide range of environmental conditions and have an ability

to grow on a variety of substrates, such as mine tailings (Lukesova 2001; Orlekowsky *et al.* 2013), barren arctic soils (Michaud *et al.* 2012), arid regions (Řeháková *et al.* 2011; Mühlsteinová *et al.* 2014) and alkaline and serpentinising springs (Blank *et al.* 2009; Suzuki *et al.* 2013). Our preliminary study documented that cyanoprokaryotes and algae are found on mafic and ultramafic soils in South Africa and that slope position plays a role in species composition. *Leptolyngbya foveolarum*, *Microcoleus vaginatus*, *Phormidium ambiguum*, *Chlamydomonas*, *Chlorococcum*, *Eustigmator magnus* as well as *Hantzschia amphioxys* were present on all three foot-slope sites at Ohrigstad, Burgersfort and Malelane (Sites 1, 6 and 7). Environmental conditions such as



Legend: Δ species; \bullet and \blacksquare sites; \rightarrow environmental variables

Axis	1	2	3	4
Eigen values	0.52	0.411	0.388	0.368
Cumulative percentage variance:				
Of species	20.2	36.2	51.3	65.6
Of environmental relations	20.2	36.2	51.3	65.6
Test of significance of first canonical axes $p = 1.0$				
Test of significance of all canonical axes $p = 1.0$				

Fig. 3. Canonical correspondence analysis (CCA) of metal concentrations and algal and cyanoprokaryote species of the different sampling sites. Abbreviations of species are given in Table 3.

an increase in soil moisture as a result of runoff and the accumulation of organic matter (Chen *et al.* 2007) could have played a role in the assembly of certain species on foot slopes. The cyanoprokaryotes *Leptolyngbya foveolarum* and *Phormidium ambiguum* were also present at the foot slope of the Mg-rich pyroxenite soil (Site 3), whereas *Microcoleus vaginatus*, *Chlamydomonas*, *Chlorococcum* and *Eustigmatos magnus* did not occur at this site. The absence of these algae as well as the low diversity observed at Site 3 could be the result of the high concentrations of Cd (0.01 mg kg^{-1}) and Hg ($0.0046 \text{ mg kg}^{-1}$) measured at this site. According to Fernandez-Piñas *et al.* (1995), Cd induces progressive disorganisation and degradation of thylakoid membranes as well as a decrease in the mobilisation of the polyphosphate granules leading to phosphorus starvation. Pinto *et al.* (2003) showed that Cd and Hg inhibit photosynthesis. However, further experimentation is clearly needed to determine whether Cd and Hg were responsible for the reduced diversity observed at this site.

Most of the cyanoprokaryotes were represented by filamentous species such as *Leptolyngbya foveolarum*, *Microcoleus vaginatus* and *Phormidium ambiguum*, and were found at all eight sampling sites. These filamentous cyanoprokaryotes can wind throughout the uppermost soil layers, forming a net-like structure that binds together soil particles (Rosentreter *et al.* 2007). This forms soil aggregates that create pathways for water infiltration and surfaces for nutrient transformations, while also increasing the soil resistance to wind and water erosion. Once the filamentous cyanoprokaryotes stabilise the soil, single-celled cyanoprokaryotes such as *Synechocystis* sp. are able to colonise the substrate (Rosentreter *et al.* 2007). The Chlorophyceae, represented by mainly coccoid species such as *Chlamydomonas* and *Chlorococcum*, were found on all the sites except at Site 3. *Chlorococcum echinozygotum* and *Chlamydomonas macrostella* were subdominant on the siliciclastic-derived soils of Sites 1 and 2, respectively. Bacillariophyceae, such as *Hantzschia amphioxys*, was found on all the sites and was subdominant at Sites 2, 4 and 5

and dominant at Site 3. According to Metting (1981), *Leptolyngbya*, *Microcoleus*, *Phormidium*, *Chlamydomonas*, *Chlorococcum* and *Hantzschia* species are all typical cosmopolitan soil algae.

Shields and Durell (1964) and Starks and Shubert (1982) suggested that the species composition of soil algal populations is affected less by the chemical nature of the substrate than by certain physical properties that influence soil moisture levels. During February 2012, when sampling took place, the study area had received between 50 and 200 mm rain, which accounts for more than 75% of the expected average summer rainfall for the area (South African Weather Service 2012). Moisture was, therefore, not a limiting factor and the chemical nature of the soils, along with other abiotic habitat features, appear to have influenced species richness and composition. In addition to the abiotic conditions such as edaphic features, biotic factors, especially the type of vegetation, can influence diversity and community composition of cryptogamic biota. For example, Rosentreter *et al.* (2007) documented that vagrant populations of *Dermatocarpon* occur on poorly drained basalt flats dominated by *Artemisia rigida*, *A. papposa*, *Antennaria flagellaris* and *Poa sandbergii*. The ecological conditions found at such sites can support completely or partially vagrant life forms in *Dermatocarpon* and other lichen genera. The differences in vegetation cover of the plant communities at the sampling sites (Sites 1–8) could have created microenvironments that favour specific algal and cyanoprokaryote species assemblages. Sites 1–7 had moderate cover, whereas Site 8 was an open savanna characterised by numerous bare soil patches (serpentine effect).

Twenty different species were identified at Site 8 (serpentine), despite this site showing elevated concentrations of heavy metals, including Co, Cr, Fe, Mn and Ni (Table 2). *Chroococcus* sp., *Scytonema ocellatum*, *Nostoc linckia*, *Chlorotetraedron* sp., *Hormotilopsis gelatinosa*, *Klebsormidium flaccidum*, *Pleurococcus* sp. and *Tetracystis elliptica* were unique to this site. Metals can be divided into those that are required by organisms in small quantities, such as Fe, Cu and Zn, which are essential in some biochemical reactions (Raven *et al.* 1999), and non-essential metals such as As, Pb, and Hg, which may cause severe harm to organisms even at very low concentrations because they do not provide any known biochemical function (Monteiro *et al.* 2012). However, the toxic effects of metallic elements on microalgae are complex and differ markedly among species, depending on the element itself and the prevailing environmental conditions (Monteiro *et al.* 2012). Stark and Shubert (1979) reported a positive correlation between Mn, N, P, silica (Si), Al, zinc (Zn) and Pb concentrations and algal abundance, and a negative correlation with Na, Cd, Cu, lithium (Li), Mo and strontium (Sr). Cyanoprokaryotes have developed efficient strategies for metal uptake and accumulation (Shcolnick and Keren 2006). According to Keren *et al.* (2002), cyanoprokaryotes are able to accumulate high concentrations of Mn in the envelope layers of their cells. Whether the high Mn supports the growth of cyanoprokaryotes or reduces competition by other groups that are less tolerant of Mn was unclear. Soil pH plays a critical role in the bioavailability of heavy metals (Rajakaruna and Boyd 2008) and, although serpentine Site 7 had high metal concentrations, the relatively higher pH at this site may have made those metals less

bioavailable (Neilson and Rajakaruna 2012). Hence, it was unclear whether the higher algal diversity at this site resulted from metal tolerance or reduced metal toxicity owing to lower metal availability. However, algae are able to grow in the presence of heavy metals as a result of a variety of tolerance mechanisms, for example, binding to cell wall, precipitation in vacuole and synthesis of heavy metal-binding compounds such as proteins, organic acids and phenolic compounds (Metha and Gaur 2005). Serpentine soils are renowned for low Ca:Mg ratio; however, DeGroot *et al.* (2005) found that low Ca:Mg ratio does not significantly explain variation in microbial community patterns; also, our study did not show a strong correlation between algal diversity and Ca:Mg. Microbes may, therefore, be responding to increased soil organic matter concentrations, probably because of the associated increases in nutrient availability and water-holding capacity.

Algae as a group are physiologically heterogeneous, making any generalisation about their soil relations difficult (O'Kelly 1974). However, favourable soil pH, moisture conditions and nutrient content contribute to species diversity and community composition (Shields and Durell 1964). The multivariate analyses did not suggest a distinct species association for serpentine sites (Sites 7 and 8). This was also true for the mafic and ultramafic sites (Sites 3, 4, 5 and 6) as well as the siliciclastic sites (Sites 1 and 2). The lack of a strong correlation with lithic type and a stronger correlation with slope position suggests that the type of slope may be more important in influencing diversity. Site pairs 1 and 2, 3 and 4 as well as 5 and 6 were sampled at different slope positions and, at times, shared similar species associations based on slope position (Fig. 3).

Conclusions

It seems unlikely that soil chemistry alone was responsible for determining species diversity and no unique algal flora for serpentine soils was confirmed. However, the serpentine soil at Kaapschehoop (Site 8) did have a unique species assemblage, comprising *Chroococcus* sp., *Chlorotetraedron* sp., *Hormotilopsis gelatinosa*, *Klebsormidium flaccidum*, *Pleurococcus* sp. and *Tetracystis elliptica*. However, this requires further investigation to determine whether it was a lithic or climatic effect.

Soil features, along with other biotic (vegetation composition) and abiotic (slope, exposure) habitat characteristics, may influence the presence and dominance of some algal and cyanoprokaryote species in harsh edaphic settings. Our results suggested that topography (i.e. slope position), rather than the chemistry of the lithic class (i.e. rock type), was most important in influencing species diversity. However, high concentrations of heavy metals also influenced species richness as well as community composition.

The characterisation of microbial communities using an isolation approach can be biased because this approach tends to favour some groups of microbes over others. Therefore, in the future, we plan to incorporate molecular approaches (Bjelland *et al.* 2011; Daae *et al.* 2013) to characterise the diversity of cyanoprokaryotes and soil algae at our sites. Additionally, the ecological heterogeneity within each site should be taken into consideration, because our sampling strategy (i.e. pooling of soil

samples) did not allow for detecting species that may be restricted to distinct microhabitats found within our sites. A composite sample does not allow for a correlation with biotic and abiotic factors, but gives a general and preliminary view on the diversity at each site. Although our findings are preliminary, the study sets the stage for detailed investigations on the relative importance of edaphic versus other habitat features on algal and cyanoprokaryote diversity and community assembly.

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