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Biological quality of a podzolic soil after 19 years of irrigated minimum-till kikuyu–ryegrass pasture

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Abstract. Conversion of natural rangeland to minimum-tillage kikuyu (*Pennisetum clandestinum*) based pastures for dairy production in the southern Cape of South Africa, may be beneficial to soil biological quality. The objective was to evaluate whether 19 years of minimum-till kikuyu-ryegrass pasture had altered the distribution and quality of biological properties formerly developed under natural rangeland. An irrigated minimum-till kikuyu-ryegrass pasture soil was compared to virgin soil with natural rangeland. Soil organic matter, soil organic C, active C, microbial biomass C, total N and enzymatic activities (β-glucosidase, urease and alkaline phosphatase) behaved similarly by having higher values in the surface layers of the cultivated pasture soil than in virgin soil, decreased with depth until they become similar at the 200–300 mm depth. Acid phosphatase activity was similar ($P > 0.05$) between soils. Vertical distribution of potentially mineralizable N was similar ($P > 0.05$) at 0–100 mm soil depth, but higher ($P \leq 0.01$) in the cultivated pasture soil than in the virgin soil. The microbial indicated along with stratification ratios for different biological indicators that the cultivated pasture soil's ecosystem functionality improved. Soil microbial functional diversity and carbon source utilisation patterns of the cultivated pasture soil and virgin soil was influenced by plant species present and root exudate composition. The soil microbial diversity, as shown by the Shannon-Weaver and Enrichment Indices, was significantly altered between cultivated pasture and the virgin soil, especially at different soil depths. A general appraisal of biological soil properties indicated that conversion of natural fynbos vegetation to irrigated minimum-till kikuyu-ryegrass pasture after 19 years of cultivation on a podzolic soil beneficial.

Additional keywords: organic carbon, enzyme activities, microbial biomass, microbial functional diversity, organic matter, total nitrogen.

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Introduction

Conversion of large areas of natural rangeland to cultivated pastures in the southern Cape region of South Africa is driven by the demand for sustainable fodder production within dairy farm systems (Botha 2003). Various management techniques have evolved to increase productivity from pastures on dairy farms. Such techniques include irrigation, fertilisation, reduced tillage, maintaining a permanent groundcover with perennial forage species, intensive grazing and incorporation of legumes (Botha 2003; Van der Colf 2011). The benefit of these management techniques resulted mainly from an increase in soil organic matter (SOM) stocks (Swanepoel and Botha 2012*a*).

Minimum-tillage systems with kikuyu (*Pennisetum clandestinum*) as a pasture base, over-sown annually with different ryegrass species (*Lolium* spp.) and varieties, have been adopted by most dairy farmers in the southern Cape region of South Africa (Van der Colf 2011). It has been reported that minimum-tillage systems may alter biological soil properties and change the distribution patterns of SOM (Müller-Stöver *et al*. 2012). Such distribution patterns are usually associated with a build-up of immobile constituents at the surface layer of minimum-tillage systems (Carter and Rennie 1982), and therefore the soil surface is mostly enriched with nutrients and SOM (López-Garrido *et al*. 2011). The distribution of SOM is of particular interest since SOM supplies nutrients to plants and microbes, maintains soil structure, and increases water-holding capacity and cation exchange capacity; SOM is therefore an important factor to ensure agro-ecosystem health

and sustainability of pastures (Conant *et al*. 2001). Changes in SOM and mineralised nitrogen (N) are associated with changes in the below-ground microbial community structure and functionality (Bossio *et al*. 2005). This is because the rate of SOM turnover is dependent on microbial functioning (Müller-Stöver *et al*. 2012), which is vital, because the microbial community safeguards ecosystem health and soil quality. Apart from changes in the distribution pattern of SOM and related parameters, the kind of vegetation grown on soil may also have a direct influence on the microbial community below the soil surface (Wardle *et al*. 2004).

Conversion of natural rangeland to permanent kikuyu-based pastures, under a minimum-tillage regime, may be beneficial to soil quality and create potential carbon (C) sinks (Conant *et al*. 2001; Desjardins *et al*. 2004). The objective of this study was to evaluate whether 19 years of irrigated, minimum-till kikuyu–ryegrass pasture on a podzolic soil altered the distribution and quality of biological properties formerly developed under undisturbed natural fynbos rangeland. In a preceding paper the effects on the physical condition of this podzolic soil were reported (Swanepoel *et al*. 2013).

Materials and methods

Site description

Two study sites which are 800 m from one another were selected on the Outeniqua Research Farm (33°58′38″S, 22°25′16″E; 204 m above sea level) near George in the southern Cape region of South Africa. These sites enabled us to compare biological indicators of soil quality between different land uses. The first site comprised an established kikuyu-based pasture and the second was characterised by fynbos vegetation in its native state. Both sites are on a Podzol (IUSS Working Group 2006) or Spodosol (Soil Survey Staff 2003) and locally known as a Witfontein soil form (Soil Classification Working Group 1991). This is one of the major soil forms in the region consisting of three diagnostic horizons: an orthic A horizon (0–200 mm), followed by a podzol B horizon (200–300 mm), which is underlain by unconsolidated material with signs of wetness (300–600 mm).

The region has a mild climate with long-term (45 years) mean ambient temperatures fluctuating in winter between 7°C and 18°C and in summer between 15°C and 25°C. No frost occurs. The long-term mean annual precipitation of the sites is 728 mm, evenly distributed throughout the year (ARC-ISCW 2012).

Experimental layout and treatments

The two sites had different land uses and hence species composition which served as treatments. At each site there were six plots. Plot size was 15 m by 15 m.

Site 1 was a 19-year-old kikuyu–ryegrass pasture on a minimum-tillage regime with permanent sprinkler irrigation. Kikuyu formed a permanent pasture base and annual Westerwold ryegrass (*Lolium multiflorum* var. *westerwoldicum*) was established into the base annually. It was over-sown once a year during March or April to increase the pasture productivity during winter and spring. Pasture was grazed to a height of 50 mm aboveground level before establishment of ryegrass. The kikuyu stubble was mulched to ground level and an Aitchison Seedmatic-3116C seeder was subsequently used to drill the seed into the mulched layer (Botha 2003; Van der Colf 2011). A strip-grazing system at a grazing intensity of ~5.7 Jersey cows ha^{-1} was used at a mean grazing cycle of roughly 30 days (Van der Colf 2011). The soil nutrient levels were maintained according to recommendations for a kikuyu–ryegrass pasture. Limestone ammonium nitrate was topdressed at an approximate rate of 55 kg N ha^{-1} on a monthly basis. Traffic intensity with a 50–55 kW tractor was restricted to the centre of a strip after grazing for N fertilisation and once a year for over-sowing ryegrass. Soil temperatures were monitored and soil water content was maintained at levels higher than ~80% of field capacity by continuous-logging soil probes (DFM Software Solutions CC 2012).

The second site remained historically undisturbed and soil was conserved in its virgin state. Natural fynbos rangeland was dominated by *Helichrysum* spp. and *Pentaschistis* spp. (Mucina and Rutherford 2006). Animals were not allowed to graze the area, which was not subject to fire.

Sampling and analyses

Annual aboveground herbage production [kg dry matter (DM) per ha] was determined on a monthly basis for the cultivated pasture before grazing by dairy cattle and that of the natural rangeland was measured seasonally. The assumption was made that the aboveground phytomass production of the natural vegetation was in equilibrium and remained stable throughout the year. Production was determined by cutting herbage within the border of quadrats (0.25 m^2) to a height of 30 mm aboveground level and dried at 60°C for 72 h.

In October 2011, representative soil samples were taken from 0–100, 100–200 and 200–300 mm depth increments in each plot for determination of particle size distribution with the hydrometer method (Day 1965) and extractable phosphorus (P) with the citric acid method (Non-Affiliated Soil Analysis Work Committee 1990). Then bulk density was measured with a hammer-driven cylindrical sampler with an inner volume of 288.8 cm^3 (Blake 1965). The measured bulk densities enabled the calculation of soil parameter stocks, which were useful to compare sites with regard to pedological significance of soil biological properties (Arshad and Martin 2002). For analysis of biological soil properties, 20 samples were taken aseptically per depth interval (0–100, 100–200 and 200–300 mm) from each plot. The SOM content was determined by gravimetric measurement of CO_2 loss during ignition at 550°C for 3 h (Broadbent 1965) and soil organic C (SOC) by dichromatic digestion using the Walkley–Black procedure (Nelson and Sommers 1982). Active C was measured by oxidation of 1.0 g oven-dry equivalent of air-dried soil with 0.02 M $KMnO_4$ in 1 M $CaCl_2$ (pH 7.2) and colourimetric measurement of non-reduced Mn at 550 nm (Weil *et al*. 2003). The microwave irradiation method was used to determine microbial biomass C (MBC) (Islam and Weil 1998) and the anaerobic incubation procedure for potential mineralisable N (PMN) determination (Drinkwater *et al*. 1996). Total soil N (total N) was analysed by Kjeldahl digestion (Bremner 1960). Soil samples destined for biological analyses were composited and stored at 4°C,

except those destined for enzyme assays. These samples were dried for 48 h at 40°C before storage.

From the measured data, microbial quotients (MBC/SOC) and C/N ratios (SOC/total N) were calculated. Stratification ratios were calculated by dividing relevant soil property values of the 0–100 mm depth interval by those of the 200–300 mm depth interval.

Enzymes involved in the C (β-glucosidase), N (urease) and P (alkaline and acid phosphatase) nutrient cycles were assayed in this study. β-Glucosidase and phosphatase activities were calculated according to methods described by Dick *et al.* (1996), determining the release of p-nitrophenyl moiety after incubation of soil with p-nitrophenyl glucoside and p-nitrophenyl phosphate, respectively. Urease activity was assayed by incubating the soil with urea according to the method of Kandeler and Gerber (1988). Residual ammonia was measured after incubation and urease activities were calculated with reference to a calibration curve.

Qualitative community level physiological profiles (CLPP) were assessed by measuring the amount of substrates and the speed of substrate utilisation (Arias *et al.* 2005). Soil samples were diluted in sterile distilled water (1 : 3000) (Buyer and Drinkwater 1997) to allow for the recovery of several types of bacteria, and to retain numerically abundant organisms while eliminating fast-growing competitors (De Fede *et al.* 2001). The soil suspensions were inoculated into the Biolog EcoPlates™ (Biolog Inc., Hayward, CA, USA) containing 31 sources of C and a control well, in triplicate. The plates were incubated at 28°C. Respiration of C sources by microbial populations reduced the tetrazolium dye within each EcoPlate well, causing a colour change. This colour change was spectrophotometrically determined twice daily over 7 days at 590 nm to determine average well colour development (Winding and Hendriksen 1997). The optical density (OD) values obtained from each plate were analysed using the average well colour development (AWCD) technique as described by Garland (1996). Standardised patterns were obtained by blanking the absorbance values for the wells with C sources against the absorbance value of the control well without a C source. Any negative values were converted to zero, and any variance in the inoculum density was accounted for by dividing the absorbance of each well by the average absorbance for the whole plate, giving the standardised OD. Instead of using the absolute values, standardised patterns were subsequently compared (Habig 2003).

The functional diversity of the soil microbial populations was determined using the amount and equitability of C substrates metabolised as indicators of richness and evenness, respectively (Garland and Mills 1991). Biodiversity was determined using the Shannon–Weaver diversity index (H′) and substrate Evenness index (E), which indicates species richness and the variation between species within the local soil microbial community, respectively (Magurran 1988).

Statistical analyses

Soil parameter data were analysed using a two sample Student's *t*-test for independent samples per depth. The data were acceptably normally distributed, but data had heterogeneous treatment variances. Data were analysed using the statistical program GENSTAT (Payne *et al.* 2011). Data on C source utilisation and enzymatic activity were subjected to non-parametric statistical analyses using STATISTICA 6.1 (StatSoft Inc. Tulsa, OK, USA). Substrate utilisation patterns were compared from the intermediate phase of the Biolog incubation and an AWCD value of 0.25 absorbance units was used as the reference point for multivariate statistical analysis of the data. Carbon substrate utilisation profiles were statistically analysed by principal component analysis (PCA) (Palojärvi *et al.* 1997) and cluster analyses (vertical hierarchical tree plots). Homogenous grouping was determined with Fisher's least significant difference (l.s.d.) at $P = 0.05$.

Results and discussion

Herbage production

The annual herbage production of kikuyu–ryegrass pasture was 20.33 kg DM ha^{-1} (s.e.m. 0.60) which was high and concurrent to the findings of Botha (2003) and Van der Colf (2011) for similar pastures in the region. However, the annual herbage yield of the natural fynbos vegetation was only 8.79 kg DM ha^{-1} (s.e.m. 0.86) and differed ($P \leq 0.001$) from that of the cultivated pasture.

Physical soil parameters

Particle size distribution for the cultivated pasture soil and virgin soil is displayed in Table 1. In both soils, clay content increased with depth, but per depth interval, the clay content was similar across land uses, except in the 200–300 mm layer. Thus, although soil biological activity may be linked to clay content (Bronick and Lal 2005; Van Antwerpen *et al.* 2009), interpretation of biological indicators should be unbiased in this study.

In both the cultivated pasture soil and the virgin soil, bulk density increased from low values in the 0–100 mm layer to high values in the 100–200 and 200–300 mm layers (Table 2). The bulk density of cultivated pasture soil was similar to that of virgin soil ($P > 0.01$).

Table 1. Mean particle size distribution (% ± s.e.m.) for cultivated pasture soil and virgin soil at three depth intervals

Soil depth	Cultivated pasture soil	Virgin soil	*P*-value
0–100 mm			
Clay	2.6 ± 0.4	2.7 ± 0.3	0.900
Silt	4.4 ± 0.7	7.0 ± 0.4	0.013
Sand	93.0 ± 0.63	90.3 ± 0.4	0.006
100–200 mm			
Clay	3.6 ± 0.4	4.0 ± 0.4	0.530
Silt	6.2 ± 0.5	8.3 ± 0.3	0.005
Sand	90.2 ± 0.5	87.7 ± 0.4	0.003
200–300 mm			
Clay	6.8 ± 0.2	5.00 ± 0.4	0.008
Silt	6.2 ± 0.7	8.00 ± 0.6	0.070
Sand	87.0 ± 0.6	87.00 ± 0.5	1.000

Organic matter indicators

Stock of organic matter indicators (Table 3) in cultivated pasture soil was higher ($P \leq 0.01$) in the upper two layers than in virgin soil, with the exceptions of MBC and PMN in the 0–100 mm layer and SOM and active C in the 100–200 mm layer, which was similar ($P > 0.01$).

In the 200–300 mm layer stock values for organic matter indicators were mostly similar ($P > 0.01$), except MBC and PMN, which were higher ($P \leq 0.05$) in cultivated pasture soil than in virgin soil. Bulk density increased as SOM related indicators decreased which was concurrent to the findings of Haynes and Tregurtha (1999). The content of SOC comprised a lower concentration of SOM (Allison 1965; Conyers *et al*. 2011) and therefore recovery of SOC for the cultivated pasture soil and virgin soil was only 50.4% and 41.4% of SOM, respectively. Conversion of virgin soil to cultivated pasture soil improved conditions supporting the build-up of SOM levels and, therefore, the soil's microbial component and soil health. Kikuyu with its high density of rhizomes and stolons is in particular a good pasture crop to enhance SOM levels (Skjemstad *et al*. 1990). Although the virgin soil had lower SOC stocks than the cultivated pasture, it maintained very high levels of SOC in its native state. The SOC is mainly regarded by agriculturalists in the southern Cape as the single most important indicator of soil quality, since there is a lack of any other indicators providing information about the condition of soil. Neither SOM nor SOC is probably the most suitable measurement to estimate biological activity for soil quality assessments, since concurrent changes to adapted management may be very slow to detect. The highly labile proportion of SOM, i.e. active C, comprised only 1.96% and 0.03% of SOM in cultivated pasture soil and virgin soil, respectively, and may be a more useful and sensitive measurement to detect subtle changes in the SOM pool than SOC concentration or stock (Karlen *et al*. 1999). The active C concentration in the cultivated pasture soil was ~530 times higher than that of the virgin soil at 0–100 mm depth, reflecting the importance of the soil surface as biologically active interface and entry point for additions of readily available organic material (López-Garrido *et al*. 2011). In these intensively grazed dairy pastures, high volumes of labile organic matter are added in forms of manure, moribund forage material and forage wastage. Active C provided additional information to that of SOC, by proving that cultivated pasture soil improved the system by introducing high volumes of vital energy substrates for microbial

Table 2. Mean bulk density (kg m^{-3} ± s.e.m.) for cultivated pasture soil and virgin soil at three depth intervals

Soil depth	Cultivated pasture soil	Virgin soil	*P*-value
0–100 mm	1280 ± 42.5	1200 ± 36.3	0.186
100–200 mm	1519 ± 54.1	1442 ± 39.4	0.271
200–300 mm	1542 ± 61.4	1512 ± 34.7	0.666

Table 3. Mean stock and concentration values (±s.e.m.) of organic matter indicators for cultivated pasture soil and virgin soil at three depth intervals

MBC, Microbial biomass C; PMN, potentially mineralisable N

Soil depth (mm)	Cultivated pasture	Virgin soil	*P*-value	Cultivated pasture	Virgin soil	*P*-value
SOM	(kg m^{-3})			(%)		
0–100	119.4 ± 5.5	71.9 ± 4.4	≤0.001	9.4 ± 0.5	6.0 ± 0.3	≤0.001
100–200	78.0 ± 5.0	68.7 ± 8.1	0.359	5.1 ± 0.3	4.7 ± 0.4	0.448
200–300	42.2 ± 2.7	65.2 ± 11.2	0.276	2.8 ± 0.1	3.7 ± 0.7	0.251
SOC						
0–100	59.2 ± 2.7	35.6 ± 1.3	≤0.001	4.6 ± 0.2	3.0 ± 0.1	≤0.001
100–200	43.2 ± 1.7	25.9 ± 1.3	≤0.001	2.8 ± 0.07	1.8 ± 0.09	≤0.001
200–300	19.7 ± 1.38	24.0 ± 1.8	0.102	1.3 ± 0.07	1.6 ± 0.09	0.030
Active C				(mg kg^{-1})		
0–100	4.19 ± 0.25	0.091 ± 0.024	≤0.001	3284 ± 194.1	76 ± 0.00	≤0.001
100–200	1.79 ± 1.07	0.11 ± 0.003	0.191	1116 ± 639.7	75.6 ± 0.00	0.105
200–300	0.24 ± 0.13	0.085 ± 0.020	0.290	150.0 ± 74.1	55.4 ± 12.75	0.275
MBC						
0–100	0.24 ± 0.079	0.19 ± 0.024	0.579	195.9 ± 71.2	158.4 ± 18.7	0.635
100–200	0.44 ± 0.055	0.18 ± 0.031	0.002	289.4 ± 31.5	127.9 ± 22.4	0.002
200–300	0.08 ± 0.027	0.16 ± 0.016	0.026	48.1 ± 16.1	104.4 ± 9.7	0.012
Total N						
0–100	5.0 ± 0.2	2.1 ± 0.05	≤0.001	0.4 ± 0.007	0.2 ± 0.006	≤0.001
100–200	3.9 ± 0.2	1.7 ± 0.07	≤0.001	0.3 ± 0.005	0.1 ± 0.006	≤0.001
200–300	1.6 ± 0.1	1.5 ± 0.2	0.558	0.1 ± 0.006	0.1 ± 0.009	0.519
PMN	(μg m^{-3} week^{-1})			(μg g^{-1} week^{-1})		
0–100	14.7 ± 2.1	11.4 ± 2.5	0.338	11.4 ± 1.3	9.5 ± 2.1	0.485
100–200	11.6 ± 2.2	1.1 ± 1.9	0.006	7.8 ± 1.7	0.8 ± 1.3	0.010
200–300	1.8 ± 0.5	–0.39 ± 0.5	0.013	1.2 ± 0.3	–0.3 ± 0.3	0.012

metabolism in the surface layer of the soil. These microbes play an important role in organic matter decomposition and nutrient cycling (Granatstein *et al*. 1987) and MBC was used to investigate the action of soil microbes within the SOM cycle (Carter 1986). Mean MBC comprised 0.41% and 0.26% of SOM in cultivated pasture soil and virgin soil, respectively. The higher MBC content in the cultivated pasture soil than in the virgin soil could be ascribed to the vigorous and large root systems of the kikuyu–ryegrass pasture with improved external environmental conditions such as water supply by irrigation, and nutrient supply by fertilisation, liming and manure from grazing animals (Carter 1986). These conditions create an environment that supports microbes by providing active C by means of root exudates and organic nutrients from manure.

The PMN revealed the capacity of the soil to supply mineralised N from SOM reserves with the aid of microbes (Edenborn *et al*. 2011). Due to low productivity of natural fynbos species and the associated low grazing capacity of 77 ha per large stock unit (Boshoff *et al*. 2001), the virgin soil required relatively low N levels to function in a sustainable manner, rendering the potential of the microbes to mineralise N higher in cultivated pasture soil than in the virgin soil. This was supported by the C/N ratios, which were narrower than 25 : 1 within all sampling layers in both soils (Table 4), a value considered the threshold for rapid mineralisation (Miles and Manson 2000).

The C/N ratio was markedly lower ($P \leq 0.01$) in the cultivated pasture soil than in the virgin soil in all sampling layers. Cultivated pasture soil had C/N ratios of ~11–12, which was in the range normally reported for agricultural soil (Karlen *et al*. 1999; Ernst and Siri-Prieto 2009). The recommended C/N ratio of a healthy SOC turnover rate in well-managed pasture is 10–12 (Miles and Manson 2000). The higher C/N ratio in virgin soil is indicative of the undisturbed and sustainable state of the ecosystem, but also reflects effects from the very low grazing capacity.

The microbial quotient, shown in Table 5, indicates the substrate-use efficiency of the microbial community and its importance in regulating SOM transformations (Moore *et al*. 2000). Insam and Domsch (1988) stated that the microbial quotient serves as an indicator of C accumulation or release.

Table 4. The C/N ratio (±s.e.m.) for cultivated pasture soil and virgin soil at three depth intervals

Soil depth	Cultivated pasture soil	Virgin soil	*P*-value
0–100 mm	11.1 ± 0.3	17.3 ± 0.4	≤0.001
100–200 mm	11.2 ± 0.3	15.8 ± 0.9	0.002
200–300 mm	12.1 ± 0.7	16.4 ± 0.7	0.002

Table 5. Microbial quotient (% ± s.e.m.) for cultivated pasture soil and virgin soil at three depth intervals

Soil depth	Cultivated pasture soil	Virgin soil	*P*-value
0–100 mm	42.1 ± 1.4	53.7 ± 6.8	0.046
100–200 mm	101.7 ± 1.1	70.6 ± 11.7	0.085
200–300 mm	36.7 ± 12.3	66.1 ± 5.7	0.046

The microbial quotient of the 0–100 and 200–300 mm depths was higher ($P \leq 0.05$) in the cultivated pasture soil than in the virgin soil, but it did not differ ($P > 0.05$) between sites in the 100–200 mm depth. Therefore, more microorganisms were sustained per unit SOM in the cultivated pasture soil than in the virgin soil.

The microbial quotient should be used as a reference point during steady-state conditions (Martens 1995). Thus, the assumption was made that the virgin soil is in equilibrium and sustainable for its relevant land use. The microbial quotient of the cultivated pasture soil was higher than ($P \leq 0.05$) or similar to ($P > 0.05$) that of the virgin soil and indicated that the cultivated soil's living component was improved. Sudden deviations from this level should indicate that the system is changing and C is being released or accumulated. It is therefore a valuable tool to predict C sequestration actions.

The degree of ecosystem functionality to 300 mm depth of the two land uses is indicated by stratification ratios (Franzluebbers 2002) in Fig. 1. A stratification ratio of 1 indicates a uniform distribution to 300 mm deep, and when >2, it generally indicates an improvement in the system (López *et al*. 1996). Stratification ratios of organic matter indicators of virgin soil remained <2, except for active C and PMN, but those of cultivated pasture soil were >2. The stratification ratios of cultivated pasture soil were higher for all organic matter indicators except for PMN. It can therefore be reasoned that the ecosystem quality and functionality were improved by enhancing organic matter indicators at the soil surface when soil is converted to cultivated pastures. Maintaining soil quality at the surface is important, since this is the interface supporting infiltration of water, gaseous exchange and organic materials from manure and forage (López-Garrido *et al*. 2011).

There was a very high positive correlation ($r = 0.94$) between SOM and SOC (Table 6), which was concurrent to the findings of Swanepoel and Botha (2012*b*). The close relationship between organic-matter related indicators was stressed by the high correlation values between SOM or SOC and the other organic-matter related indicators, except for MBC.

Enzyme activities

The activities of four soil enzymes were assayed to evaluate ecosystem functioning and are shown in Table 7. β-Glucosidase, urease and alkaline phosphatase activities were higher ($P \leq 0.01$) in the cultivated pasture soil than in the virgin soil in all layers, except for β-glucosidase at 200–300 mm depth. The enzymatic activities rapidly declined with soil depth in the cultivated pasture soil, which is the trend usually observed (Dick *et al*. 1988; Curci *et al*. 1997; Green *et al*. 2007) and is concomitant with the decline in organic matter indicators observed, also noted by Verhulst *et al*. (2010). Acid phosphatase activity had a different distribution pattern than the other soil enzymatic activities. The virgin soil and the cultivated pasture soil had similar ($P > 0.05$) activities in all depths. Soil pH(KCl) of the virgin soil was significantly lower ($P \leq 0.05$) at all soil depths than that of the cultivated pasture soil, which rendered the efficiency of acid phosphatase higher in virgin soil (Table 8).

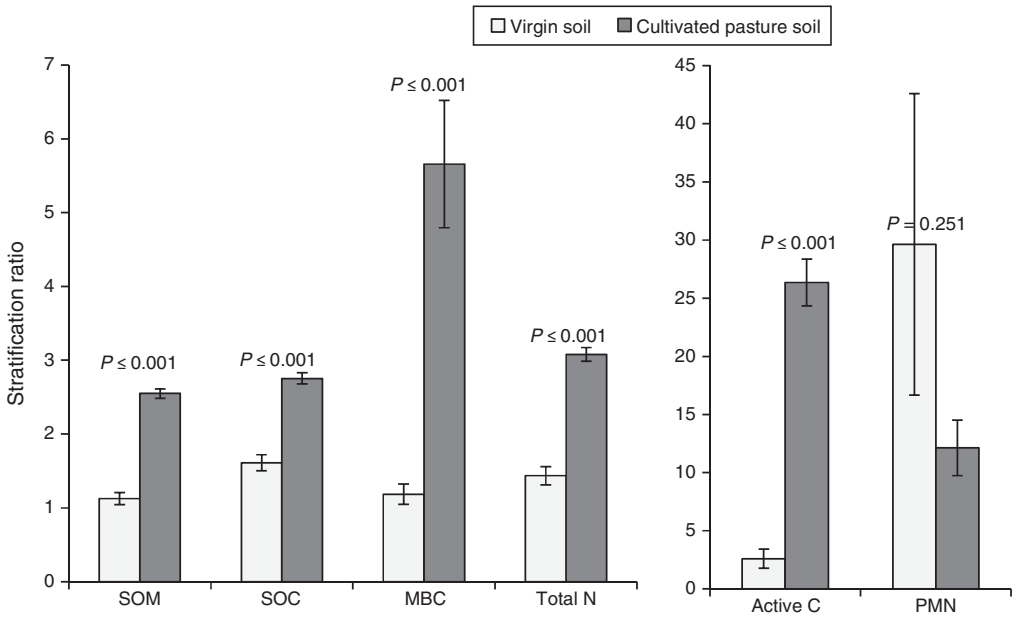

Fig. 1. Stratification ratios of soil organic matter (SOM), soil organic carbon (SOC), microbial biomass C (MBC) and total N (left); and active C and potentially mineralisable N (PMN) (right). Capped lines indicate standard error.

Table 6. Pearson's correlation coefficients (*r*) for soil organic matter (SOM), soil organic carbon (SOC), active C, microbial biomass C (MBC), total N, potentially mineralisable N (PMN), C/N ratio and microbial quotient at a significance level of $P = 0.01$

n.s., Not significant ($P > 0.01$)

	SOM	SOC	Active C	MBC	Total N	PMN	C/N	Microbial quotient
SOM	1							
SOC	0.94	1						
Active C	0.80	0.83	1					
MBC	0.59	0.64	0.52	1				
Total N	0.89	0.97	0.86	0.65	1			
PMN	0.71	0.80	0.65	0.64	0.29	1		
C/N	–0.30	–0.36	–0.47	–0.38	–0.58	–0.38	1	
Microbial quotient	0.24n.s.	0.27	0.18n.s.	0.87	0.78n.s.	0.34	–0.25n.s.	1

This agreed with the findings of Dick (1992), who reported a decrease in acid phosphatase activity when native soil is cultivated. This effect was also visible from the negative but strong correlation between acid phosphatase and SOC, β-glucosidase, alkaline phosphatase and urease activity (Table 9).

The patterns of SOM and SOC distribution and β-glucosidase, alkaline phosphatase and urease activity were similar ($P > 0.01$), but stratification ratios were generally higher ($P \leq 0.01$) than those of organic matter indicators (Fig. 2). β-Glucosidase activity had a very high correlation ($r = 0.94$) with SOC, since it is involved in the C cycle. Although urease and alkaline phosphatase are not directly involved in C turnover, the correlations between them and SOC were also high ($r = 0.90$ and 0.88, respectively), proving the important indirect functions of SOM in the P and N cycles. Correlation between urease and total N was very high ($r = 0.95$), but a correlation between alkaline phosphatase and P content was moderate ($r = 0.60$).

Stratification ratios >2 were reported for β-glucosidase, urease and alkaline phosphatase activities in the virgin soil, which was not the case of the organic matter indicators. However, for β-Glucosidase and urease, the cultivated pasture soil had much higher stratification ratios ($P \leq 0.01$) than the virgin soil. It could therefore be speculated that the thresholds for stratification ratios of enzymatic activities should be higher to indicate improvement of the system. Availability of C sources at the various depths and, therefore, microbial activity should influence the different enzymatic activities. The correlation between enzymatic activities and MBC were, however, moderate to low ($r = 0.59$, Table 9).

Functional diversity

The mechanism of colour development in Biolog EcoPlates is associated with differences in C-source utilisation relating to the

number of viable microorganisms with the ability to utilise the substrates within the EcoPlate wells as a sole C source. The results of this research also confirm findings by Garland and Mills (1991) that the direct incubation of environmental samples in EcoPlates produced patterns of metabolic response useful in the characterisation of soil microbial communities. From Fig. 3, it is clear that the functional diversity differed between the virgin soil and the cultivated pasture soil.

In accordance with results found by Bissett *et al*. (2011), results obtained in this study indicated no significant ($P > 0.05$) differences in overall CLPP between sites or sampling depths. Observed changes in overall CLPP can be attributed to the very different plant community composition, as well as the quality and quantity of SOM available with increased soil depth (Wardle *et al*. 2004) in the two sites. The composition of pasture crops on cultivated soil altered the functional composition of the responsive soil microbial community, as determined by the composition of root exudates, which is greatly influenced by the crop present (Bardgett *et al*. 1999; Stephan *et al*. 2000). The difference in root-exudate composition between crops thus contributed to the difference in physiological profiles of soil microbial populations between the sites. The released root exudates attract microbial populations that are especially well adapted to utilise the specific compounds very rapidly (Garbeva *et al*. 2004). Utilisation of substrate guilds by soil microbial communities in the virgin soil and cultivated pasture soil at three depths is illustrated in Table 10.

Table 7. Enzyme activities (μg g^{-1} h^{-1} ± s.e.m.) for cultivated pasture soil and virgin soil at three depth intervals

Soil depth (mm)	Cultivated pasture soil	Virgin soil	*P*-value
	β-glucosidase		
0–100	4751 ± 168.8	1582 ± 70.8	≤0.001
100–200	2018 ± 147.6	854 ± 65.1	≤0.001
200–300	739 ± 65.5	596 ± 67.4	0.168
	Urease		
0–100	269 ± 18.8	33.3 ± 3.3	≤0.001
100–200	122 ± 14.8	20.0 ± 1.3	0.002
200–300	52.6 ± 2.2	14.9 ± 2.2	≤0.001
	Alkaline phosphatase		
0–100	5437 ± 802.3	711 ± 92.7	0.004
100–200	1950 ± 285.4	502.0 ± 53.3	0.006
200–300	655 ± 165.2	244.3 ± 76.5	0.040
	Acid phosphatase		
0–100	1019 ± 52.9	1217 ± 75.6	0.069
100–200	1295 ± 53.0	1398 ± 37.7	0.137
200–300	1430 ± 25.1	1365 ± 52.7	0.325

Table 8. pH(KCl) (±s.e.m.) for cultivated pasture soil and virgin soil at three depth intervals

Soil depth	Cultivated pasture soil	Virgin soil	*P*-value
0–100 mm	5.5 ± 0.04	4.1 ± 0.05	≤0.001
100–200 mm	5.4 ± 0.04	4.3 ± 0.06	≤0.001
200–300 mm	5.6 ± 0.04	4.4 ± 0.02	≤0.001

No significant differences ($P > 0.05$) in utilisation patterns of all substrate guilds were observed at the same depths between the two sites, except for carboxylic acid utilisation at 100–200 mm and esters and amines at the 200–300 mm depth. Results clearly indicated that carboxylic acids and amino acids were more readily utilised in the cultivated pasture soil, whereas the remainder of the substrate guilds were more readily utilised in the virgin soil. It appears that substrate guilds were equally utilised in the cultivated pasture soil, irrespective of depth. Virgin soil, on the other hand, showed significant ($P \leq 0.05$) differences in carbohydrate, ester and amine utilisation at increased soil depth.

Cluster analysis for the soil microbial functional diversity in the cultivated pasture soil and the virgin soil is illustrated in Fig. 4. Cluster analysis assigns treatments into groups, so that treatments in the same cluster are more similar to each other than to treatments in other clusters. Distinctive clusters could be observed between CLPP in the cultivated pasture soil and in the virgin soil. Carbon source utilisation in the cultivated pasture soil was more similar at 0–100 and 100–200 mm depths, compared with 200–300 mm depth. The virgin soil demonstrated a slightly different pattern, in which substrate utilisation was more similar at 100–200 and 200–300 mm depths than at 0–100 mm depth. The distinctive difference in clustering between the two sites could largely be attributed to higher SOM, SOC, active C, MBC and total N values in cultivated pasture soil, which decreased with depth until values became comparable in the 200–300 mm soil layer.

Biodiversity indices

Soil microbial diversity was determined by the Shannon–Weaver diversity index, which distinguished between the two

Table 9. Pearson's correlation coefficients (*r*) for soil organic carbon (SOC), total N, microbial biomass C (MBC), extractable P and enzymatic activities at a significance level of $P = 0.01$

	SOC	Total N	Extract. P	MBC	β-glucosidase	Urease	Alk. phase	Acid phase
SOC	1							
Total N	0.97	1						
Extractable P	0.54	0.67	1					
MBC	0.64	0.65	0.44	1				
β-glucosidase	0.94	0.96	0.61	0.59	1			
Urease	0.90	0.95	0.69	0.54	0.96	1		
Alkaline phosphatase	0.88	0.90	0.60	0.56	0.94	0.94	1	
Acid phosphatase	–0.77	–0.72	–0.24	–0.46	–0.77	–0.68	–0.71	1

sites' soil microbial communities based on the number of different C sources utilised in Biolog EcoPlates. Values of this index typically range from 1.5 to 3.5, and rarely increase to >4.5 (Magurran 1988). A moderate to high percentage of C sources were utilised, with average values of 2.7 and 2.6 in cultivated pasture soil and virgin soil, respectively (Tables 11 and 12).

From Table 11 and Table 12, it is clear that a higher soil microbial diversity was present in the cultivated pasture soil than the virgin soil. Since the Shannon–Weaver index is based on the amount of C sources utilised, it is clear that the amount of available C sources declined with depth, i.e. a decline in microbial diversity. No difference ($P \leq 0.05$) in microbial diversity was observed between depths in the cultivated pasture soil (Table 11). A significant difference ($P \leq 0.05$), however, could be observed in the virgin soil between the 0–100 mm depth with the highest microbial diversity, and the 100–200 and 200–300 mm depths with lower diversity. This observation might be attributed to the absence of any soil disturbance to distribute the top-layer SOM to the lower depths to make it more readily available to the microbial communities at levels deeper than 100 mm.

The Evenness (equitability) index (E), a derivative of the Shannon–Weaver index, was used as an indication of equality of abundance of species within a soil microbial population. Substrate Evenness indices ranged between 0.8 and 0.9 (Table 11). According to Magurran (1988), substrate evenness assumes a value between 0 and 1, with 1 presenting a situation in which all species are equally abundant. This results in less variation in microbial populations between species, therefore, less dominance and higher diversity. Soil microbial species in the cultivated pasture soil were more equally abundant within the microbial community, compared with the virgin soil. The significant decrease ($P \leq 0.05$) in species abundance, resulting in increased species dominance, at different depths in the virgin soil, as well as between the various depths in the virgin soil compared with corresponding depths in the cultivated pasture soil (Table 12), suggested an increase in particular microbial species within the virgin soil specialising in the utilisation of specific C sources released by the high diversity of fynbos vegetation present at the site. This observation is also supported by the lowest soil microbial diversity present in the virgin soil, i.e. different soil microbial species within microbial populations within the virgin soil became less equally abundant,

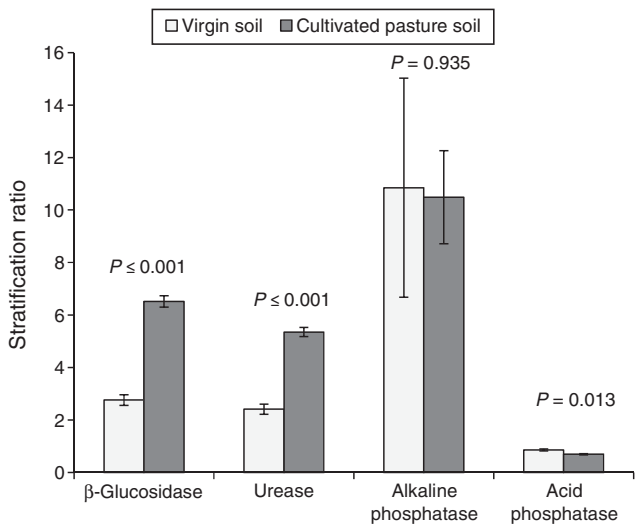

Fig. 2. Stratification ratios of enzyme activity. Capped lines indicate standard error.

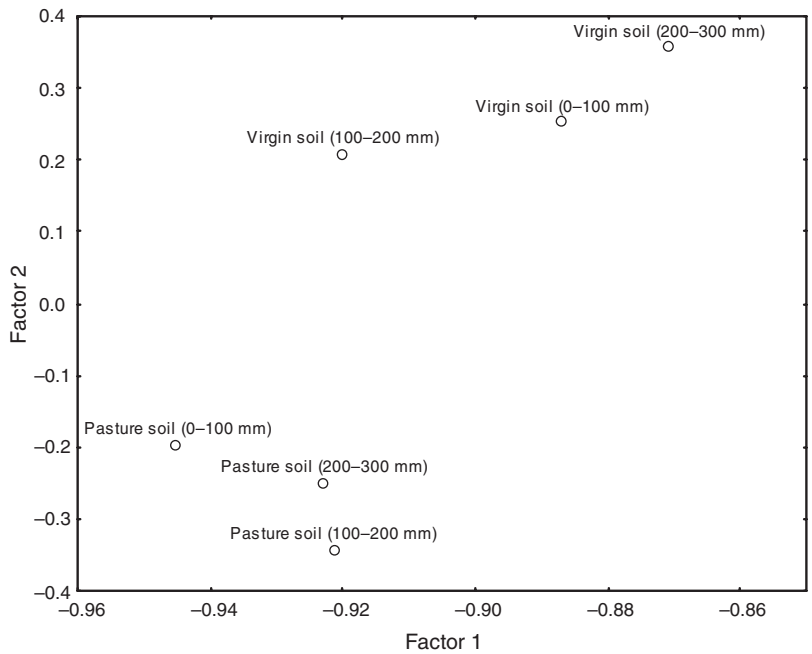

Fig. 3. Principal component analysis plot of community-level physiological profiles of cultivated pasture soil and virgin soil at 0–100, 100–200 and 200–300 mm depths.

Table 10. Carbon source utilisation in virgin soil and cultivated pasture soil at three sampling depths
Within columns, means followed by the same letter are not significantly different at $P = 0.05$

Soil depth (mm)	Carbohydrates	Carboxylic acids	Amino acids	Polymers	Esters	Phosphorylated chemicals	Amines
			Virgin soil				
0–100	9.27 ± 0.96b	7.67 ± 0.96ac	5.14 ± 0.58a	4.86 ± 0.56b	1.62 ± 0.29a	1.73 ± 0.77ab	0.71 ± 0.19a
100–200	8.39 ± 0.60ab	6.41 ± 0.83c	6.63 ± 0.83a	3.15 ± 0.95ab	2.24 ± 0.62ab	2.96 ± 0.89b	1.22 ± 0.47ab
200–300	6.16 ± 0.93a	8.71 ± 0.98abc	6.02 ± 0.86a	3.09 ± 0.93ab	3.03 ± 0.46b	1.78 ± 1.02ab	2.21 ± 0.34b
			Cultivated pasture soil				
0–100	8.34 ± 0.76ab	9.80 ± 0.76ab	5.47 ± 0.72a	3.01 ± 0.45ab	1.26 ± 0.19a	2.01 ± 0.53ab	1.12 ± 0.35a
100–200	8.05 ± 1.33ab	9.90 ± 0.59ab	6.80 ± 0.86a	2.64 ± 0.59a	1.42 ± 0.22a	1.23 ± 0.46ab	0.96 ± 0.28a
200–300	6.50 ± 1.48ab	10.21 ± 0.93b	6.86 ± 0.75a	3.64 ± 0.66ab	1.85 ± 0.46a	0.86 ± 0.31a	1.07 ± 0.51a

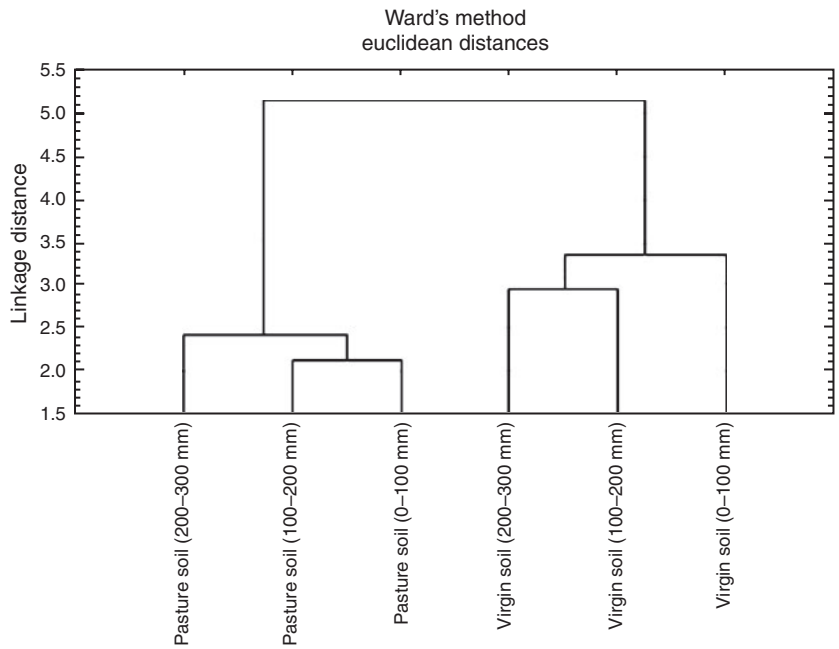

Fig. 4. Dendrogram illustrating the clustering of community level physiological profiles of cultivated pasture soil and virgin soil at 0–100, 100–200 and 200–300 mm depths.

Table 11. Shannon–Weaver diversity and Evenness indices (±s.e.m.) illustrating soil microbial species richness and variation, respectively, at increasing depth in the cultivated pasture soil and virgin soil
Within columns, means followed by the same letter are not significantly different at $P = 0.05$

Soil depth (mm)	Shannon (H')	Evenness (E)
	Cultivated pasture soil	
0–100	2.84 ± 0.06a	0.88 ± 0.01a
100–200	2.68 ± 0.10a	0.84 ± 0.02a
200–300	2.62 ± 0.13a	0.87 ± 0.02a
	Virgin soil	
0–100	2.73 ± 0.03b	0.83 ± 0.01b
100–200	2.54 ± 0.07a	0.80 ± 0.01ab
200–300	2.57 ± 0.06a	0.78 ± 0.02a

Table 12. Shannon-Weaver diversity and Evenness indices (±s.e.m.) illustrating soil microbial species richness and variation, respectively, at increasing depth between the cultivated pasture soil and virgin soil
Within columns, means followed by the same letter are not significantly different at $P = 0.05$

	Shannon (H′)	Evenness (E)
0–100 mm		
Virgin soil	2.73 ± 0.03a	0.83 ± 0.01a
Cultivated pasture soil	2.84 ± 0.06a	0.88 ± 0.01b
100–200 mm		
Virgin soil	2.54 ± 0.07a	0.80 ± 0.01a
Cultivated pasture soil	2.68 ± 0.10a	0.84 ± 0.02a
200–300 mm		
Virgin soil	2.57 ± 0.06a	0.78 ± 0.02a
Cultivated pasture soil	2.62 ± 0.13a	0.87 ± 0.02b

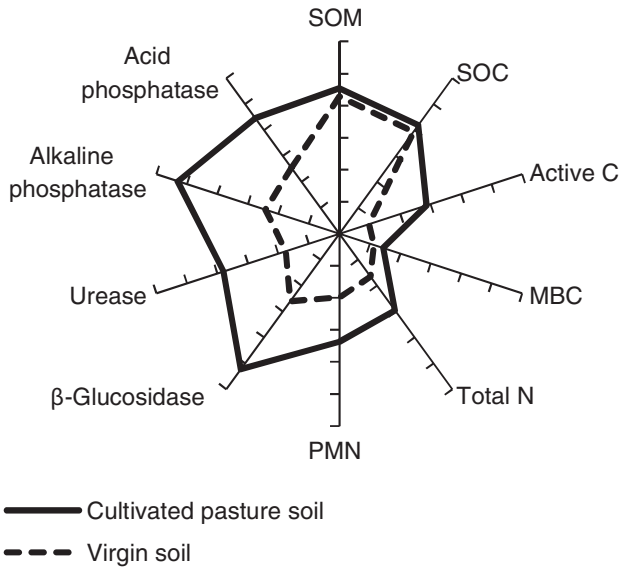

Fig. 5. Star plot of the measured biological parameters of virgin soil and cultivated pasture soil. SOM, Soil organic matter; SOC, soil organic C; MBC, microbial biomass C; PMN, potentially mineralisable N.

but more specialised, thus dominating the virgin soil. The difference in microbial diversity between the two sites might also be attributed to the irrigation of the cultivated pasture soil, whereas the fynbos site is solely dependent on rainfall.

Soil health appraisal

Figure 5 represents a star plot of the virgin soil and cultivated pasture soil. The area of the stars may be interpreted as the biological activity and vigour of the soil. It is clear that the cultivated pasture soil had higher biological activity and vigour than the virgin soil, and conversion of natural vegetation to cultivated pasture improved soil health and markedly modified the biological component of the soil.

Conclusion

The importance of SOM to maintain soil health of a sandy podzolic soil and a balance between environmental sustainability and agricultural production is emphasised by biological soil properties. Conversion of virgin soil to irrigated cultivated pasture soil improved environmental conditions, supported the build-up of SOM levels and, in effect, enhanced the soil's microbial component, ecosystem functionality and soil health. This was reflected by the distribution patterns, stratification and degree of organic-matter indicator accumulation and enzymatic activities. Soil microbial functional diversity in the cultivated pasture soil and virgin soil was greatly influenced by plant species present and root exudate composition. The soil microbial diversity between cultivated pasture soil and the virgin soil was significantly altered, especially at different soil depths. The plant diversity present influenced the composition of root exudates, thus contributing to the difference in microbial diversity. The released root exudates attracted microbial species that were well adapted to utilise the specific compounds very rapidly. A general appraisal of biological soil properties indicated that conversion of natural fynbos vegetation to irrigated, minimum-till, kikuyu–ryegrass pasture after 19 years of cultivation on a podzolic soil is beneficial. The indicator values reported may also serve as baseline values, since such reference values for the southern Cape region of South Africa are not available; however, the initial data from soil quality studies on the Outeniqua Research Farm near George in South Africa should offer the best reference values and can be amended with future research.

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