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REGULAR ARTICLE

Microbial responses to P addition in six South African forest soils

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Abstract Forests growing on highly weathered soils are often phosphorus (P) limited and competition between geochemical and biological sinks affects their soil P dynamics. In an attempt to elucidate the factors controlling the relative importance of these two sinks, we investigated the relationship of between soil microbial growth kinetics and soil chemical properties following amendments with C, N and P in six South African forest soils. Microbial growth kinetics were determined from respiration curves derived from measurements of CO_2 effluxes from soil samples in laboratory incubations. We found that microbial growth rates after C + N additions were positively related to NaOH-extractable P and decreased with soil depth, whereas the lag time (the time between substrate addition and exponential growth) was negatively related to extractable P. However, the growth rate and lag time were unrelated to the soil's sorption properties or Al and Fe contents. Our results indicate that at least some of the NaOH-extractable inorganic P may be biologically available within a relatively short time (days to weeks) and might be more labile than previously thought. Our results also show that microbial utilization of C + N only seemed to be constrained by P in the deeper part of the soil profiles.

Keywords Phosphorus availability · Microbial bioassay · Weathered soils · Soil respiration · Microbial growth rate · Hedley fractionation

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Introduction

Plant growth in highly weathered soils with high phosphorus (P) sorption capacities and low P concentrations is generally P limited (Sanchez 1976; Yost et al. 1979). In such soils, phosphorus sorption restricts both plant and microbial P availability, but microbes

may be able to access soil P that is unavailable to plants in the short-term (Cross and Schlesinger 1995). Microbial P uptake is an important biological sink (Magid et al. 1996; McLaughlin et al. 1988; Oberson et al. 2001; Stewart and Tiessen 1987) and can occur as rapidly as P sorption, even when strong sorption sites are present (Olander and Vitousek 2004). Microbial utilization and mobilization of P may also be important for plant nutrition, because plants can benefit from microbial uptake of P, via mycorrhizal associations, or via P mobilization by the activities of rhizosphere organisms (Chen et al. 2004; George et al. 2006; Smith and Read 1997; Whitelaw 2000).

In terms of the strength of the biological sink, microbial growth is usually constrained by carbon (C) availability (Gallardo and Schlesinger 1990; Wardle 1992), but it can be co-limited by P, especially in soils with high concentrations of C, aluminium (Al) and iron (Fe) (Duah-Yentumi et al. 1998; Oberson and Joner 2004). In such soils, high microbial demand for P may strongly influence the partitioning of P between biological versus geochemical sinks. However, the availability of carbon-containing substances that can be used as sources of energy also strongly affect P dynamics. For instance, Olander and Vitousek (2004) found that tracer P moved from the sorbed pool into the microbial pool, when microbial P demand was stimulated by the addition of C in a laboratory study. In nature, similar stimulation by C may occur in the rhizosphere when root exudation occurs, or at the surface of mineral soil where there is fresh litter.

In highly weathered soils, such as oxisols, P sorption is correlated with amorphous Fe and Al oxides (Freese et al. 1992; Loganathan et al. 1987), and concentrations of Al and Fe are likely to be high throughout their profiles. The geochemical sink in highly weathered soils may thus constrain P availability throughout the soil profile. Meanwhile, biological sinks, notably microbes, may be constrained by carbon inputs and may therefore take up P more strongly in the upper parts of a soil profile because of the higher local C contents. In less weathered soils, such as spodosols in temperate and boreal climates, Wood et al. (1984) suggested that the biological and geochemical sinks are separated by depth, due to the formation of a mineral B-horizon that acts as a geochemical sink separated from the more biologically active upper soil horizons. In highly weathered soils, there is no clear separation of the biological and geochemical sinks (Agbenin 2003) with depth. However, the relative importance of these two sinks may still differ with depth, due to potential differences in carbon inputs.

Microbial growth in highly weathered soils has been studied using respiration measurements (Cleveland et al. 2002; Duah-Yentumi et al. 1998; Gnankambary et al. 2008; Ilstedt et al. 2003). Such measurements (in combination with experimental amendments of C, N and P) have provided information on the relative importance of added substrates for microbial growth, and hence on the factors influencing the biological sink (Cleveland et al. 2002; Duah-Yentumi et al. 1998) and microbial P availability (Gnankambary et al. 2008; Ilstedt et al. 2003; Teklay et al. 2006). However, the cited studies have all focused on surface soils and there have been no investigations, to our knowledge, on depth-related variations in microbial growth responses to substrate additions. Hence, analyses of the relationship between microbial growth and soil depth could provide further valuable information on interactions between variables such as C, Al and Fe concentrations and the activities of soil microorganisms. Given that plants may benefit from the microbial uptake and/or mobilization of P, such information may significantly aid our understanding of plant P acquisition

To unravel the relationships between vertical variations in soil chemical properties that contribute to the strength of geochemical sinks and microbial growth responses to C, N and P additions (biological sinks), we selected six forest plantation soils in South Africa of varying types and properties. The growth responses of microbes in each of these soils (at various depths) were estimated from respiration measurements in laboratory incubations. This approach enabled us to examine changes in microbial growth responses with depth in the highly weathered soils examined and the effects on microbial growth of inherent variations in measured soil chemical properties—including contents of specific P pools extracted by the Hedley fractionation procedure (Hedley et al. 1982)—and the availability of C, N and P. In addition, a specific hypothesis was tested: that in accordance with the importance of C supplies noted above, the inherent soil C content, rather than P sorption, is the main factor affecting microbial responses to C, N and P additions.

Materials and methods

Study site and soil sampling

We selected six forest plantation sites in South Africa for the study: two with sandy soils—Gauge Farm and Compartment H7 Salpine (Salpine) located in Kwambonambi, Coastal Zululand on the Mozambique coastal plains—and four with clay soils located in the KwaZulu-Natal Midlands region at Karkloof, Westfield, Pinewoods and Honey Grove. Details of the sites (geographic coordinates, vegetation and mean annual rainfall and temperature) and their soils are presented in Table 1.

The six sites were sampled in December 2004. At each of the study sites, a soil pit was excavated and ca. 1 dm^3 soil samples (spanning ca. 0.1 m vertically) were collected from the pit wall in December 2004 at selected depths in the soil profile, as listed in "Appendix 1". Soil samples were taken down to at the most 0.6 m in the clay soils and 1.4-m in the sandy soils. Saprolite prevented deeper soil sampling in the clay soils. In the sandy soils, the selection of sampling depths was based on morphological changes in the soil profile. The samples were placed in polyethylene plastic bags, gently homogenized, kept at 5°C then frozen within 12 h of sampling at −20°C until further analysis.

Freezing of soils is likely to cause lysis of some microbial cells. However, freezing was unavoidable because the alternative of storing samples for a prolonged time at temperatures higher than 0°C would have caused even more detrimental effects to the microbial community (Stenberg et al. 1998; Verchot 1999). In order to minimize the effects of freezing during the microbial bioassays, no substrates were added until a stable CO_2 efflux from the samples was observed (2 days to 3 days). We also assume that the relative differences between the soils were not affected by freezing, since previous studies using frozen soils collected from sub-tropical environments, using techniques similar to those applied in this study, have not given any indication that freezing samples confounds the interpretation of results (Gnankambary et al. 2008; Ilstedt et al. 2003; Teklay et al. 2006).

Microbial respiration responses to C, N and P additions

Microbial responses were tested using a microbial bioassay, in which CO_2 evolution was measured hourly, largely as described by Nordgren (1992), using a respirometer (Respicond V, Nordgren Innovations, Djäkneboda, Sweden) to trap carbon dioxide (CO_2) respired from samples in prepared microcosms by potassium hydroxide (KOH). The setup can be used to monitor respiration kinetics in detail (Marstorp and Witter 1999) and has been used to study microbial responses to additions of C, N and P (Demetz and Insam 1999; Giesler et al. 2004; Ilstedt et al. 2003; Teklay et al. 2006) in various soils.

Table 1 Information on the location, climate, vegetation and soil at the six sampled South African forest plantation sites

Site	Coordinates	Mean annual		Vegetation (plantation year)	Parent material	Soil texture	Soil type
		rainfall (mm)	temp (°C)				
Gauge Farm	28°65′ S 32°06′ E	1084	21.7	*Eucalyptus grandis*, *E. urophylla* (2000)	Aeolian sand deposit	sand	Entisol
Salpine	28°57′ S 32°26′ E	1223	21.7	*E. grandis*, *E. urophylla* (1998)	Aeolian sand deposit	sand	Entisol
Karkloof	29°41′ S 30°20′ E	896	16.0	*E. grandis* (1998)	Dolerite	clay	Oxisol
Westfield	29°37′ S 30°07′ E	988	14.8	*E. smithii* (1998)	Dolerite	clay	Oxisol
Pinewoods	29°66′ S 30°08′ E	942	15.7	*Pinus patula* (1992)	Mudstone	clay	Oxisol
Honey Grove	29°37′ S 30°77′ E	760	17.0	*P. taedea* (1979–1984)	Arenite	clay	Oxisol

To prepare the microcosms, duplicate portions of soil from each sample were placed in each of two 250 ml plastic vessels and mixed with 0.73 g perlite. The use of perlite as a substrate carrier is a modification by Ilstedt et al. (2007) of the original method by Nordgren (1988, 1992), introduced because perlite has a high water holding capacity and allows larger additions of glucose relative to soil, due to a dilution effect that reduces osmotic effects. The larger additions of glucose are needed to prevent the glucose from being depleted before the P in the soil. Perlite is a porous heated volcanic material, which does not change the bioavailability of added nutrients and by itself does not result in any detectable change in respiration after C + N + P additions (Ilstedt et al. 2007). For the clay soils, amounts equivalent to 0.20 g organic matter (OM; determined from loss on ignition, 5 h at 550°C), but smaller amounts of carbon (0.10 g or 0.05 g OM) were present in the duplicate sandy soil samples, since they had much lower C contents, and it would have been impossible to add soil with as much OM. The dry weight soil contents of the clay and sandy soil microcosms were ca. 1.3 g and 13 g, respectively. The water content of both substrates in the vessels was adjusted to −20 kPa, determined with suction plates in order to optimize the moisture conditions for microbial growth (Ilstedt et al. 2000).

The microcosms were then incubated at 20°C, and when respiration had been stable for 30 h, glucose and N (C + N) were added in excess—2 g g^{-1} OM glucose and 0.325 g g^{-1} OM $(NH_4)_2SO_4$—in a powdered form to all samples to ensure that C and N were not limiting to the microbial community (Nordgren 1992). To one of each pair of duplicate vessels, a small amount of P (0.57 mg g^{-1} OM P as NaH_2PO_4) was also added in a 100 μL solution. Thus, two amendment treatments (C + N and C + N + P) were applied to each pair of duplicate samples.

For each sample, the basal respiration rate was determined by calculating the average of 30 hourly measurements taken prior to the glucose and nutrient addition (Fig. 1), which was followed by an immediate increase in respiration rate or "substrate induced respiration" (SIR) (Anderson and Domsch 1978). This increased respiration rate persists until the microorganisms start to grow exponentially. The exponential growth rate (μ) is calculated as the slope of the log-transformed respiration rate plotted against time. In addition, lag time, defined as the time between substrate addition and exponential growth, was calculated as the time between substrate addition and the time when the slope of the log respiration rate versus time regression line intercepts log SIR (Nordgren 1988). Since glucose and N are added in excess, the maximum respiration rate is assumed to occur at the time (tmax) when the available P pool in the native soil is exhausted (Nordgren 1992). We define microbial P availability as the increase in the rate of microbial activity (growth rate) when other nutrients are added in excess, and hence P is limiting (Giesler et al. 2004), estimated here by the observed increases in μ following C + N additions. Thus, this is not an absolute, but rather a relative measure of the microbial P availability. Giesler et al. (2004) have shown that both lag time and tmax are related to the P availability of soils and can be used in a similar way as the microbial growth rate.

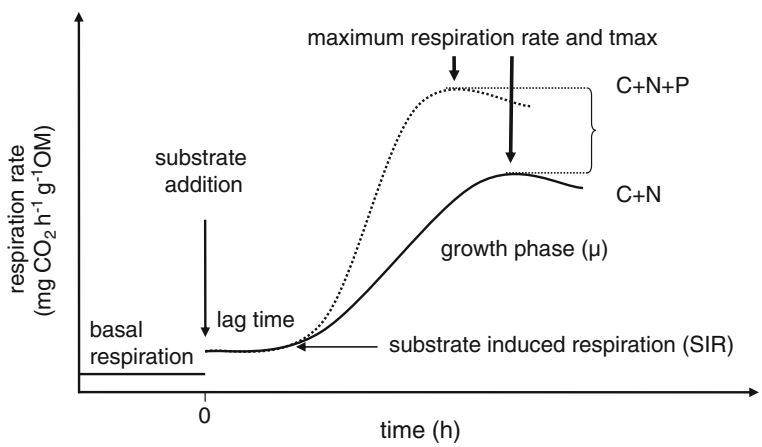

Fig. 1 A model of soil respiration responses before and after substrate additions; glucose and nitrogen (C + N) with and without a small dose of phosphorus (P). The ΔC parameter represents the increment in CO_2 evolution after a small P addition. Basal respiration, SIR, lag time, growth rate and maximum respiration rate and tmax are defined in the text

Extractable pools of P, Al and Fe

Sequential extractions have been widely used to characterize soil P availability by applying a series of increasingly strong extractants, the assumption being that the different fractions also reflect differences in P availability (see Cross and Schlesinger 1995). Here, specified pools of phosphorus in the soil were analyzed following extraction by the Hedley sequential extraction procedure (Hedley et al. 1982), as described by (Binkley et al. 2000), except that an anion exchange membrane was used instead of an iron-oxide impregnated filter strip, following Saggar et al. (1990). For these extractions approximately 10 g (dw) of each field-moist soil sample was placed in a 250 ml centrifuge bottle. In each sequential step, an extracting solution was added, the samples were agitated on an orbital shaker (18 h, 150 rpm) and then centrifuged (15 min, 14,000 rpm, 10°C). In the first step, 180 ml de-ionized water and an anion exchange membrane with an area of approximately 4.5×8.5 cm (55164 2S, BDH Laboratory Supplies, Poole, England) were added to the centrifuge bottle. After extraction on the shaker, soil on the membranes was rinsed back into the centrifuge bottles with de-ionized water. The centrifuge bottles were centrifuged and the supernatant removed, leaving the soil pellets at the bottom of the bottles. The membranes were then eluted with 40 ml 0.5 M NaCl (Saggar et al. 1990) in 150 ml bottles, which were shaken for 1 h. In the subsequent soil extraction steps, 180 ml 0.5 M $NaHCO_3$, 0.2 M NaOH and 1.0 M HCl were sequentially added to the soil pellets. After the last extraction step, the remaining soil was washed with 180 ml de-ionized water, shaken for 1 h, centrifuged and the supernatant discarded prior to air-drying. The residual P fraction remaining after the extractions was released by acid digestion with 10 ml H_2SO_4 (soil-solution ratio 1:5) and H_2O_2 as a catalyst in a block digester (360°C).

The first two extraction steps, which involved use of an anion exchange membrane and extraction with $NaHCO_3$ solution, are designed to extract labile inorganic and organic P fractions. The third step, with NaOH extraction, is assumed to extract Al and Fe surface-bound inorganic P and partially stabilized organic P in soil organic matter. In the fourth step (1.0 M HCl), inorganic P in calcium phosphates and inorganic P occluded within Al and Fe oxides is assumed to be extracted (Cross and Schlesinger 1995). The remaining residual fraction contains mainly recalcitrant P.

Al and Fe concentrations in the soil samples were determined following parallel extraction with 0.1 M sodium pyrophosphate ($Na_4P_2O_7$) and 0.2 M acid oxalate ($C_2H_8N_2O_4$), each adjusted to pH 3 (Buurman et al. 1996). About 2 g dw of field-moist soil were weighed into 180 ml plastic bottles, extracted on an orbital shaker for 18 h (sodium pyrophosphate) or 4 h in darkness (oxalate), at a soil-solution ratio of 1:40. The extracts were filtered (00H, Munktell Filter AB, Grycksbo, Sweden) and stored frozen (−20°C) until analysis. The oxalate extracts both organically bound and amorphous Fe and Al oxides, whereas pyrophosphate mainly extracts organically bound Fe and Al. Total C and N in soil samples were determined after drying (70°C, 3 days) and milling with a ball mill.

Phosphate sorption index

To characterize the phosphate sorption capacity of the soils, a single point P sorption method was used (Bache and Williams 1971). The amount of P sorbed from an application of 125 μmol g^{-1} (dw) (3.9 g kg^{-1}) was called the P sorption index of the soil defined as:

$$x/\log c$$

where x is the amount of P sorbed and c is the equilibrium P concentration in the solution. A high index indicates that the tested soil has a high P sorption capacity. Two grams (dw) of soil were weighed into a 60 mL flask and 50 mL of de-ionized water were added. Phosphate was then added as KH_2PO_4. The suspension was shaken for 24 h, filtered (Munktell 00H filter paper, pore size about 1 μm; Grycksbo, Sweden) and analysed for P immediately after the filtration. The amount of sorbed P (x) was calculated as the difference between the initial and final phosphate concentration after 24 h of incubation.

We also calculated the P sorption capacity using the empirical relationship between oxalate-extractable (Al and Fe) and sorbed P, following Borggaard et al. (2004), who found that the experimentally determined phosphate sorption capacity, was well predicted by the function:

$$P_{calc} = 0.22 \times Al_{oxalate} + 0.12 \times Fe_{oxalate} + 0.02 \times (Fe_{dithionithe} - Fe_{oxalate})$$

in a large range of non-calcareous soils. We ignored $Fe_{dithionithe}$, since in analyses of two Tanzanian soils Borggaard et al. (2004) found that excluding this parameter resulted in only 1% lower estimates of P_{calc}. A simplification of the degree of phosphorus saturation (DPS, van der Zee and van Riemsdijk 1988) was calculated (P_{sat}) as the percentage molar ratio between oxalate extractable P and the sum of oxalate extractable Al and Fe .

$$P_{sat} = (P_{oxalate}/(Al_{oxalate}+Fe_{oxalate})) \times 100$$

Chemical analyses

Phosphorus analyses of the extracts from the Hedley fractionation were performed using a flow injection analyzer (5012 Analyzer, Tecator, Höganäs, Sweden), as follows. All extracts were analysed for phosphate. The $NaHCO_3$ and NaOH extracts were also analyzed for total P. Prior to analysis, the $NaHCO_3$ and NaOH extracts were filtered (Millex-HV 0.45 μm, Millipore, Molsheim, France), diluted 1:5 and 1:10, respectively, to a volume of 5 mL) and amended with 20 μL sulphuric acid.

The supernatants in the $NaHCO_3$ and NaOH extracts were coloured; thus, the measured P concentrations were adjusted by subtracting the apparent P concentrations in blanks prepared separately using corresponding matrices, but without the P reagents. Total P contents in the $NaHCO_3$ and NaOH extracts were then determined following acidified potassium persulphate digestion. Organic P contents in these two extracts were calculated as the difference between their determined total P and phosphate contents.

Pyrophosphate and oxalate extracts were analysed for Al and Fe (and P for oxalate extracts) by inductively coupled plasma optical emission spectra using a Varian Vista Ax Pro instrument. Total C and N in soil samples were measured using a Perkin Elmer Elemental CHNS analyzer.

Statistics

Initially, the relationships between the chemical and biological variables of interest were examined using pooled data for all of the soils. However, it was apparent that the properties of the clay and sandy soils strongly differed, thus the correlations between their measured variables were subsequently analyzed separately. The two sample data sets were sandy soils (Gauge Farm and Salpine; N=11) and clay soils (Karkloof, Westfield, Pinewoods and Honey Grove; N=20). The two data sets were tested for relationships between microbial parameters after C + N additions (dependent variables) and various P fractions (independent variables), using stepwise linear regression (SPSS 12.0.1 software, Chicago, Illinois, USA). The variables were entered when the probability of F was less than or equal to 0.05 and removed when it was greater than or equal to 0.10. The various P fractions (dependent variables) were also analysed for their relationships with carbon content (C %) and oxalate- and pyrophosphate-extractable Al and Fe (independent variables) using the same statistical test. Uncertainties are reported as 95% confidence intervals.

Results

Relationships between P fractions and C, al and Fe

Overall, the average total P concentration was five to 15 times higher in the clay soils than in the sandy soils (Table 2). Carbon and extractable Al and Fe concentrations were also considerably higher in the clay soils than in the sandy soils ("Appendix 1"). Total P decreased with depth in all soil profiles except the sandy Salpine (Table 2). Carbon and oxalate-extractable Al and Fe concentrations also generally declined with depth ("Appendix 1").

Inorganic P (the sum of all inorganic P fractions) accounted for on average 9% of total P and consistently less than 30% of total P (all soils combined). Inorganic P was generally highest at the surface and not detectable in the deeper layers of the sandy soils (Table 2). In the clay soils, inorganic P was dominated by NaOH-extractable inorganic P, averaging 91% of inorganic P, while in the sandy soils, inorganic P was dominated by membrane P, averaging 57% of inorganic P. Clay soils were dominated by residual P, which accounted for 62% of the total P on average. The second most abundant fraction was the NaOH-extractable organic P fraction, which accounted for, on average, 25% of total P (Table 2). In contrast, NaOH-extractable organic P dominated in sandy soils, averaging 50% of total P. Residual P comprised 27% of total P on average in

Table 2 Concentrations of extracted phosphorus fractions in the sampled soils

Site	Depth	Membrane P	$NaHCO_3$ P_i	$NaHCO_3$ P_o	NaOH P_i	NaOH P_o	HCl P_i	Residual P	Total P
	(m)	(mg kg^{-1})							
Gauge Farm	0–0.1	1.2	0.0	9.1	0	26.4	2.9	27.2	66.9
	0.1–0.2	0.2	0.2	8.1	0	22.2	0.2	19.2	50.1
	0.2–0.3	0.2	0.0	8.4	0	24.3	0.2	9.7	42.8
	0.8–0.9	0.1	0.0	7.8	0	17.6	0.0	10.0	35.5
	1.2–1.3	0.1	0.0	7.4	0	15.2	0.1	17.2	39.9
	1.3–1.4	0.1	0.0	0.0	0	15.3	0.0	9.1	24.4
Salpine	0–0.1	1.2	0.0	8.7	6.8	19.7	1.7	9.8	47.8
	0.1–0.2	0.3	0.0	8.1	0	21.9	0.4	5.6	36.3
	0.2–0.3	0.2	0.0	8.2	0	19.5	0.1	0.8	28.8
	0.5–0.6	0.2	0.0	8.2	0	19.3	0	5.4	33.1
	0.7–0.8	0.1	0.0	7.7	0	16.3	0.2	11.1	35.3
Karkloof	0–0.1	0.5	21.6	0	50.6	112.0	0	303.2	487.9
	0.1–0.2	0.1	0	11.3	30.4	77.0	0	210.9	329.7
	0.2–0.3	0.1	9.9	0	18.1	63.5	0	155.1	246.7
	0.3–0.4	0.1	4.4	3.8	12.1	50.7	0	120.6	191.7
	0.4–0.5	0	6.5	1.3	4.4	52.7	0.1	121.7	186.8
Westfield	0–0.1	0.2	0	23.0	74.5	157.5	2.3	662.7	920.3
	0.1–0.2	0.1	0.7	12.7	48.6	120.8	1.5	653.4	837.8
	0.2–0.3	0	4.6	6.2	10.9	115.3	0.2	502.1	639.4
	0.4–0.5	0	2.5	7.0	3.4	85.3	0	216.3	314.5
	0.5–0.6	0	0	7.8	4.7	77.6	0	175.1	265.2
Pinewoods	0–0.1	0.1	1.0	18.8	68.7	109.5	0.1	274.2	472.4
	0.1–0.2	0.1	1.9	11.0	58.3	88.1	0.2	197.1	356.7
	0.2–0.3	0.1	0.2	10.4	30.6	71.3	0	154.4	267.0
	0.3–0.4	0.1	0	10.4	21.4	80.8	0.3	209.9	322.9
	0.4–0.5	0.0	0	9.2	16.9	80.6	0.1	192.3	299.2
Honey Grove	0–0.1	0.2	0	20.0	63.4	60.8	0	135.1	279.5
	0.1–0.2	0.1	0	14.1	63.4	41.5	0	101.7	220.7
	0.2–0.3	0.1	2.5	7.8	40.2	40.7	0	88.1	179.4
	0.3–0.4	0.1	6.6	2.5	25.8	42.4	0	87.1	164.4
	0.4–0.5	0	3.3	5.3	23.6	39.7	0	108.2	180.2

P phosphorus; P_i inorganic P; P_o organic P

sandy soil, whilst $NaHCO_3$-extractable organic P accounted for 19% in the sandy soils (Table 2).

The two independent variables that were most strongly correlated to the soils' concentrations of various P fractions and total P, according to stepwise linear regression analysis, were their carbon and oxalate extractable Al contents (Table 3). Oxalate-extractable Fe and pyrophosphate-extractable Al and Fe were also related to P, in some cases (Table 3). Positive correlations were found between the P fractions and: C, pyrophosphate-extractable Al and oxalate-extractable Al and Fe. The only variable found to be significantly negatively correlated with the P fractions was pyrophosphate-extractable Fe. In clay soils, the concentrations of most P fractions were linearly related to C, Al and Fe, whereas in sandy soils this was only true for membrane P and NaOH-extractable organic P.

Table 3 Stepwise linear regression with various phosphorus forms as the dependent variables. The independent variables are oxalate- (Ox) and pyrophosphate- (Pyr) extractable aluminium (Al) and iron (Fe) and carbon content (C%). Sandy soils: Gauge Farm and Salpine. Clay soils: Karkloof, Westfield, Pinewoods, and Honey Grove. Only significant models are shown

Soil type	Dependent variable	Model	R_{adj}^2	*p*-value
Sand	Membrane P	Membrane $P=0.058\times C\%-0.001\times Al_{ox}$	0.82	$p=0.001$
	NaOH P_o	NaOH $P_o=0.075\times Fe_{ox}+0.51$	0.36	$p=0.039$
	HCl P_i	HCl $P_i=0.10\times C\%$	0.50	$P=0.014$
Clay	Membrane P	Membrane $P=0.001\times C\%-0.00002\times Al_{ox}+0.001$	0.72	$p<0.001$
	NaOH P_i	NaOH $P_i=0.11\times C\%-0.011\times Fe_{pyr}+0.004\times Al_{pyr}+0.88$	0.76	$p<0.001$
	NaOH P_o	NaOH $P_o=0.007\times Al_{ox}+0.18\times C\%+0.47$	0.86	$p<0.001$
	HCl P_i	HCl $P_i=0.0003\times Al_{ox}-0.0003\times Fe_{ox}-0.03$	0.63	$p<0.001$
	Residual P	Residual $P=0.039\times Al_{ox}+0.95\times C\%-4.07$	0.92	$p<0.001$
	Total P	Total $P=0.07\times Al_{ox}+1.21\times C\%-0.04\times Fe_{ox}-3.73$	0.95	$p<0.001$

P phosphorus; P_i inorganic P; P_o organic P

Phosphate sorption

The P sorption capacity of the sandy soils was very low (P sorption indices, 0–7; Table 4). The P sorption indices for the clay soils were considerably higher, ranging from 41 to 261, with an average of 105 (Table 4). The P sorption index was strongly positively related to the oxalate-extractable Al and Fe concentrations of the soil (Fig. 2). Similarly, the sorbed P was also strongly positively related to oxalate-extractable Al and Fe ($R^2=0.87$, $p<0.001$). The calculated Langmuir maximum of the phosphate sorption isotherm (Pcalc) and the measured sorbed P were also positively related ($R^2=0.87$, $p<0.001$). The P sorption was relatively uniform with depth in three of the clay soils, with somewhat larger variation in the Westfield soil (Table 4).

Microbial respiration responses to C, N and P additions

Basal respiration and the maximum respiration rate (mg CO_2 g^{-1} dw, following C + N addition) were positively and linearly related ($R^2=0.73$, $P<0.001$) and both showed a positive linear relationship with the carbon content in the soil ($R^2=0.83$ and 0.84 respectively; $P<0.001$).

The variations in microbial responses to C, N and P additions with depth are illustrated for the Westfield soil in Fig. 3. On average, it took 100±44 h after C + N addition for the microorganisms' exponential growth phase to start, 491±155 h to reach the maximum respiration rate, and the lag time increased with sampling depth in the incubations of samples from all soil profiles ("Appendix 2"). Lag time and time to maximum respiration rate both decreased, by 45% and 58% on average, respectively, when P was added together with C + N.

All soils responded to the addition of P (C + N + P treatment), though the magnitude of the response differed considerably between sampling depths, with the lag time and time to reach maximum respiration showing more than 10-fold differences (Fig. 3, "Appendix 2"). The exponential growth rate decreased with sampling depth in all clay soils, but not for the sandy soils (Salpine and Gauge Farm; "Appendix 2"). The slope of the exponential growth was up to 26 times higher for samples that received C + N + P, than for samples that only received glucose and N additions ("Appendix 2", Fig. 3).

Relationships between microbial respiration kinetics, P forms and P sorption

Microbial response variables were significantly related to soil P (Table 5). Their growth rate was best explained by the NaOH-extractable inorganic P fraction in the clay soils (Table 5), but no significant relationship was found between these variables in incubations with the sandy soils. Lag time and time to reach maximum respiration showed a negative relationship in incubations of samples of both clay and sandy soils. We found no correlation between the P sorption index and the growth rate (Fig. 4), lag time or time to reach maximum respiration. For instance,

Table 4 Degree of phosphorus (P) saturation (P_{sat}), P index, adsorbed P (Pads) and calculated P sorption maximum (Pcalc). The P_{sat} is calculated as the ratio between oxalate-extractable P and the sum of oxalate-extractable aluminium and iron. The P index is calculated as the ratio between sorbed P and the log concentration after a single P amendment. A higher value indicates a higher sorption capacity

Site	Depth (m)	P_{sat} (%)	P index	Pads	Pcalc
				mg P kg^{-1}	
Gauge Farm	0–0.1	6.9	0	0	43
	0.1–0.2	5.0	0	0	38
	0.2–0.3	4.3	0	0	58
	0.8–0.9	4.4	6	130	21
	1.2–1.3	4.0	0	0	11
	1.3–1.4	7.0	0	0	11
Salpine	0–0.1	6.5	0	0	43
	0.1–0.2	4.1	1	12	58
	0.2–0.3	2.7	0	0	86
	0.5–0.6	2.4	0	0	81
	0.7–0.8	1.4	7	137	78
Karkloof	0–0.1	0.7	64	1086	1105
	0.1–0.2	0.4	99	1498	1292
	0.2–0.3	0.2	80	1289	1125
	0.3–0.4	0.2	84	1322	895
	0.4–0.5	0.1	86	1354	830
Westfield	0–0.1	1.0	213	2201	3291
	0.1–0.2	0.8	113	1609	2831
	0.2–0.3	0.5	267	2343	3135
	0.4–0.5	0.4	203	2141	2704
	0.5–0.6	0.3	114	1617	1468
Pinewoods	0–0.1	0.8	118	1652	1691
	0.1–0.2	0.6	98	1634	1660
	0.2–0.3	0.5	99	1486	1641
	0.3–0.4	0.4	115	1620	1621
	0.4–0.5	0.3	124	1713	1592
Honey Grove	0–0.1	1.2	41	769	846
	0.1–0.2	0.9	47	862	862
	0.2–0.3	0.6	45	811	785
	0.3–0.4	0.4	42	788	715
	0.4–0.5	0.3	46	835	718

P_{sat} was calculated as the ratio between oxalate-extractable P and the sum of oxalate extractable Al and Fe

the growth rate following the C + N addition was clearly lower in incubations with the surface soil from Westfield than in incubations with the lowermost soil layer (Fig. 3), although the P sorption index was almost twice as high in the former than in the latter.

Discussion

The Hedley fractionation procedure has been widely used to estimate plant P availability (Gahoonia and Nielsen 1992; Hedley et al. 1982; Schmidt et al. 1996; Tate et al. 1991). However, few studies have related microbial respiration kinetics to the various Hedley fractions (Ilstedt et al. 2003), despite microorganisms playing a crucial role in soil P dynamics (Oberson et al. 2001; Oehl et al. 2001; Olander and Vitousek 2004). Our results suggest that, at least for clay soils, the NaOH-extractable inorganic P fraction is an important predictor of microbial growth rate. The results also suggest that microbial respiration responses to C + N or C + N + P additions are constrained by factors other than surface sorption of phosphate. The results also show that there are depth-related variations in respiration responses.

The general decrease with depth in microbial growth rate indicates that the microbial P availability is highest in the surface layers of soils and decreases with depth. Previous studies have shown that P additions in combination with C + N induce increases in microbial growth rates (Giesler et al. 2004; Ilstedt and Singh 2005; Nordgren 1992) that are linearly proportional to the amount of added P (Demetz and Insam 1999). The growth rate has also been shown to be positively related to plant P availability (Giesler et al. 2002, 2004). Thus, given C and N in excess, the microorganisms in the surface soils could probably utilize the available P more rapidly than those in the

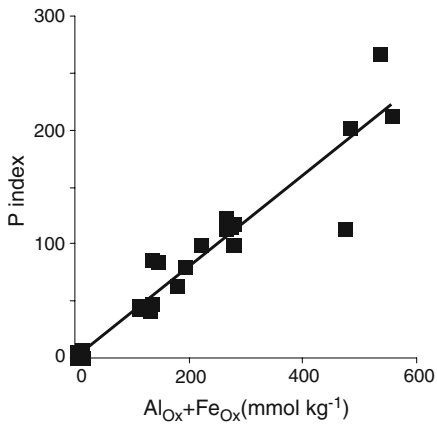

Fig. 2 The relationship between phosphorus sorption index (P index) and the sum of oxalate-extractable aluminium and iron (Al_{Ox} + Fe_{Ox}). The relationship can be expressed as P index= 0.40×(Al_{Ox}+Fe_{Ox})+0.47; R^2=0.95, p<0.001

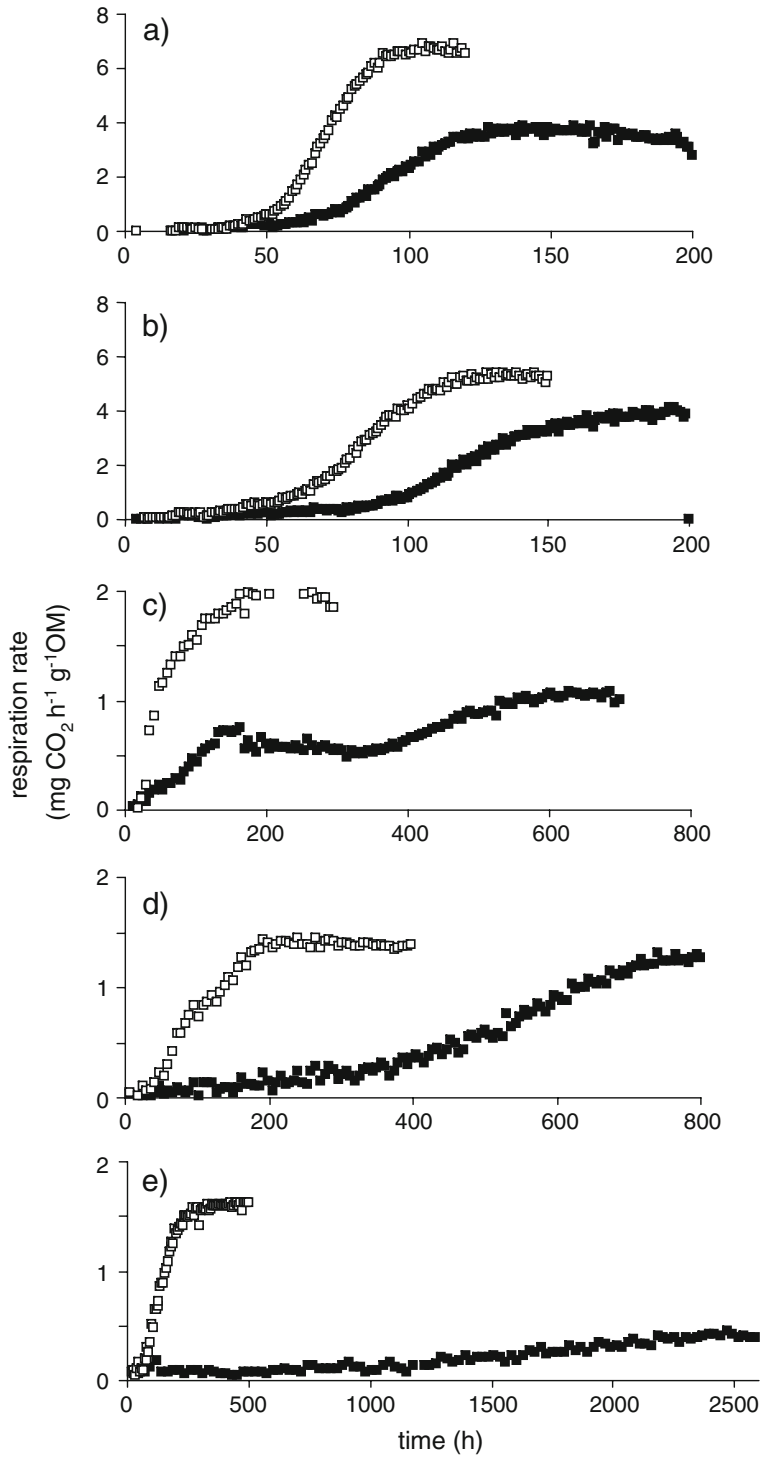

Fig. 3 Respiration curves after substrate additions to samples obtained from indicated depths in a soil profile (Westfield). The open squares denote C + N + P additions and the filled squares denote C + N additions. The soil layers are a) 0–0.1-m, b) 0.1–0.2-m, c) 0.2–0.3-m, d) 0.4–0.5-m and e) 0.5–0.6-m. Note the different scales for the x-axes

deeper soil layers. This is also consistent with the shorter lag time and time to reach maximum respiration observed in incubations with samples from the surface layers, than in incubations with samples from the deeper layers ("Appendix 2"). The lower growth rates observed in the latter cannot be explained by surface sorption of phosphate, since the P sorption indices we calculated suggest that the P

Table 5 Results from stepwise linear regression between microbial growth parameters (following C + N additions, as defined in the text) as dependent variables and various P fractions (independent ariables). Entered independent variables are: membrane-P, NaHCO3 (extractable) P_i, NaHCO3 P_o, NaHCO3 P, NaOH P_i, NaOH P_o, NaOH P, HCl P_i, residue P and total P. P_i=inorganic P; P_o=organic P. NaOH P is the sum of NaOH P_i and P_o and $NaHCO_3$ P is the sum of $NaHCO_3$ P_i and P_o. Sandy soils: Gauge Farm and Salpine. Clay soils: Karkloof, Westfield, Pinewoods and Honey Grove

Soil type	Dependent variable	Model	R_{adj}^2	*p*-value
Sand	Lag time	Lag time=−308×NaOH P+268	0.58	p=0.004
	Growth rate	n.s		
	tmax	tmax=−988×NaOH P+1045	0.60	p=0.003
Clay	Lag time	Lag time=−110×NaOH P_i+238	0.27	p=0.011
	Growth rate	Growth rate=0.008×NaOH P_i	0.56	P<0.001
	tmax	tmax=−447×NaOH P_i+1028	0.37	p=0.003

P phosphorus; P_i inorganic P; P_o organic P

sorption capacity is higher in at least some of the upper soil layers and is generally high throughout the clay soils.

Given P, in addition to C and N, the microbial populations in the samples from the deeper soil layers responded rapidly, with more pronounced increases in growth rates and reductions in lag time than those in samples of the surface layers. The rapid response in the deeper layers shows that these layers support an active microbial community that can respond to nutrient amendments. However, the response to C and N seems to be strongly limited by P in the deeper soil layers and much less so in the surface layers. We suggest that this is related to the availability of P. The microbial community in the deeper layers probably differs from that in the surface soil, and could use the added C + N less efficiently. However, this seems unlikely to be the main cause of the observed differences since samples from both surface and deeper layers responded rapidly following P addition when the same C + N sources were supplied. Phosphorous-limited boreal forest humus soils with extremely high Al and Fe contents also show similarly rapid growth rate responses when P is added, in combination with C + N (Giesler et al. 2004). In the boreal forest soils, observed growth responses were clearly related to the sorption properties of the soils. In the soils examined here, however, differences in the growth responses between surface and deeper soil layers were unrelated to either Al and Fe contents or P sorption.

The microbial growth rate was best predicted by the NaOH-extractable P, indicating that this fraction is an important source of P for microbes. The NaOH-extractable inorganic P was the dominant inorganic P fraction in the clay soils, accounting for 85% of the inorganic P pool, on average, whereas membrane P contributed less than 1%. The correlation between the two fractions most likely reflects a sorption equilibrium between the surface-bound NaOH-extractable inorganic P pool (Cross and Schlesinger 1995) and the membrane-extractable P. Microorganisms have a known phosphate-solubilising capacity (Whitelaw et al. 1999). There is also experimental evidence that they can utilize phosphate sorbed to variably charged minerals, such as goethite and amorphous Al-oxide (He and Zhu 1998). Our results are consistent with these findings and may explain the positive relationship between the microbial growth rate and NaOH-extractable P. It also seems likely that the reductions with depth in inorganic P are related to the apparent decrease in microbial P availability.

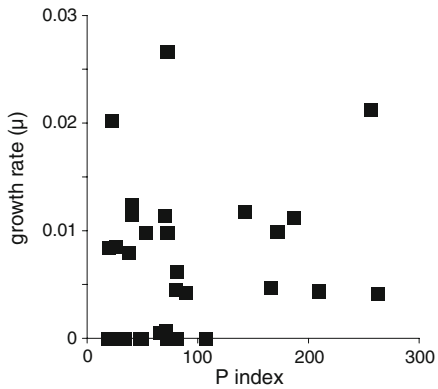

Fig. 4 Relationship between the growth rate following glucose and nitrogen amendments and the phosphorus sorption index (P index). A higher P index value indicates a larger phosphorus sorption capacity

A number of previous studies have stressed the importance of organic P as a plant or microbial P source (Gahoonia and Nielsen 1992; George et al. 2002; Oberson et al. 2001; Richardson et al. 2005 and references therein; Stewart and Tiessen 1987; Tarafdar and Claassen 1988). Accordingly, we also found the microbial growth rate to be significantly related to $NaHCO_3$-extractable organic P in the clay soils (data not shown), but it was not significantly related to any of the other organic P forms. This could be because $NaHCO_3$-extractable organic P is assumed to be a readily available form of organic P. Our results are in accordance with results of a recent study by McDowell et al. (2008), who found that the correlation between plant P uptake and extractable P strengthened when organic P was considered in addition to inorganic P. The absence of a significant relationship between microbial growth rate and other fractions of organic P may be explained by a difference in cost efficiency of microorganisms using organic versus inorganic P, both of which can have strong sorption affinities to different Al and Fe forms (Anderson and Arlidge 1962; Anderson et al. 1974; Celi et al. 2003). Using either P form may thus require a breakage of P surface binding. This can be promoted by the exudation of organic acids (Hinsinger 2001), which incurs C costs to the microorganisms. However, the release or solubilisation of inorganic P may also be promoted by proton release, and thus does not necessarily involve a C cost (Illmer et al. 1995). In contrast, the utilization of organic P will always require C (and N) for the production of enzymes and thus incur larger C (and N) costs, compared with the utilization of inorganic P. It may thus be more cost efficient for heterotrophic microorganisms to utilize inorganic P rather than organic P It should be stressed, however, that in surface soils with fresh C inputs via litter and rhizosphere deposition, C may not be a constraint for microorganisms. In the deeper soil, C constraints are probably more important.

The soil C content in the surface soil layers was always highest in the top 0.1 m of soil. Carbon is likely to be more readily available here than in the deeper soils layers, due to fresh inputs from litterfall and possibly higher rates of rhizodeposition. Microbial activity was also higher with an increased C content, as indicated by the strong correlation between basal respiration and soil C, in accordance with previous findings that glucose additions to soils increase microbial P uptake (Schmidt et al. 1997). Further support for a connection between soil organic matter inputs and microbial P availability have been provided by incubation experiments with P-sorbing Ultisols, in which C + N additions decreased P sorption and increased microbial P and the labile fractions of soil P (Lee et al. 1990). Microbial P availability has also been found to be higher under tree canopies (where the organic content, but not total P, is reportedly higher than in open areas) in agricultural soils in Burkina Faso (Gnankambary et al. 2008). Thus, although the causal relationships between the variations in microbial P availability and C are complex, and not fully understood, qualitative differences in the organic C and P pools may play an important role. The soil C to N and C to organic P ratios we found indicate that there are qualitative differences in soil organic matter between soil depths ("Appendix 1"). Similar qualitative changes in organic P and C with depth in tropical soils have also been reported (Möller et al. 2000).

The sandy soils we examined differed from the clay soils in having lower P, Al and Fe concentrations and very low P sorption capacities. The results indicate that organic P must be the dominant P form utilised by microbes in the sandy soils. The increases in lag time and time to reach maximum respiration observed with increases in sampling depth in the incubations of these soils are thus most likely related to qualitative differences in the organic P pool with depth. This hypothesis is also supported by the decrease in C to organic P ratio with depth. The increase in the maximum respiration rate following P additions was also stronger than in incubations of samples of the clay soils, indicating that the microbial communities could be more strongly P-limited in the sandy soils, in accordance with the previous discussion regarding the higher C costs of utilizing organic P.

The addition of P decreased the average lag time from about 4 to 1.5 days. Lag time provides an indication of how rapidly the microorganisms can switch from catabolism to anabolism, and our results suggest that P additions decreased the time required for the microbes to start exponential growth. This would require rapid initial utilization of the added P, and tracer studies using ^{32}P have shown that microbial uptake can be rapid and that this process can efficiently compete with sorption by geochemical sinks (Olander and Vitousek 2004; 2005). Olander

and Vitousek (2004) also found that up to 70% of added P was taken up by microorganisms within 30 min of addition and that microbial immobilisation was further increased by C additions. Thus, the decrease in lag time noted in our soil incubations was probably due to rapid microbial P immobilisation. Ilstedt et al. (2007) varied the soil to added P ratio in soils with high Al + Fe contents, in similar bioassays to those in our study, and found the enhancement of the microbial response following C + P + N addition, compared with C + N alone, to be independent of the soils' Al + Fe content, which also supports the contention that microbes can utilise the added P, even if much has been sorbed before uptake.

Conclusions

In summary, our results clearly indicate that P can be a co-limiting nutrient for microbial growth along with N and C, and that glucose-utilizing microorganisms can be constrained by P availability. This seems to especially true in the deeper parts of the soil profiles and less so in the surface layers. When the soils are amended with P, the microorganisms have the capacity to utilize the added P rapidly and this appears to be unrelated to the soils' sorption properties. Our results show that the NaOH-extractable inorganic P fraction is related to microbial growth. This indicates that at least some of the NaOH-extractable inorganic P may be available within a relatively short timeframe (days to weeks) and thus is more labile than previously thought. Our findings confirm our hypothesis that microbial P utilization is unrelated to sorption in these highly weathered soils. We suggest that microbial P availability is primarily driven by the availability of labile organic carbon.

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Appendix 1

Soil characteristics for six South African soil profiles.

Site	Depth	C%	N%	P_o%	C/N	C/P_o	Al_{Ox}	Fe_{Ox}	Al_{Pyr}	Fe_{Pyr}
	(m)						mmol kg^{-1}			
Gauge Farm	0–0.1	0.62	0.04	0.006	16	99	5	2	15	5
	0.1–0.2	0.21	0.03	0.005	7	42	5	2	5	1
	0.2–0.3	0.24	0.03	0.004	8	57	7	2	23	6
	0.8–0.9	0.06	0.01	0.004	6	17	3	1	0	0
	1.2–1.3	0.04	0.01	0.004	4	10	1	1	5	2
	1.3–1.4	0.04	0.02	0.002	2	16	1	1	7	1
Salpine	0–0.1	0.72	0.05	0.004	14	189	5	3	9	2
	0.1–0.2	0.47	0.03	0.004	16	132	7	4	19	4
	0.2–0.3	0.43	0.05	0.003	9	151	11	3	24	3
	0.5–0.6	0.31	0.02	0.003	16	94	12	1	68	3
	0.7–0.8	0.15	0.02	0.004	8	43	11	1	65	4
Karkloof	0–0.1	9.94	0.51	0.042	19	239	141	39	264	131
	0.1–0.2	5.46	0.32	0.030	17	182	153	67	304	190
	0.2–0.3	4.80	0.26	0.022	18	220	131	63	327	207

(continued)

Site	Depth	C%	N%	P_o%	C/N	C/P_o	Al_{Ox}	Fe_{Ox}	Al_{Pyr}	Fe_{Pyr}
	(m)						mmol kg^{-1}			
	0.3–0.4	2.99	0.19	0.018	16	171	116	28	381	244
	0.4–0.5	2.15	0.12	0.018	18	122	108	26	453	277
Westfield	0–0.1	10.17	0.53	0.084	19	121	395	162	495	181
	0.1–0.2	9.02	0.51	0.079	18	115	346	128	639	246
	0.2–0.3	5.81	0.31	0.062	19	93	368	169	496	231
	0.4–0.5	2.93	0.16	0.031	18	95	294	188	499	319
	0.5–0.6	1.44	0.11	0.026	13	55	157	106	252	163
Pinewoods	0–0.1	5.45	0.30	0.040	18	135	210	70	420	136
	0.1–0.2	3.90	0.23	0.030	17	132	204	73	316	117
	0.2–0.3	3.65	0.21	0.024	17	155	195	85	253	114
	0.3–0.4	3.20	0.19	0.030	17	106	197	76	388	180
	0.4–0.5	2.53	0.16	0.028	16	90	196	69	373	167
Honey Grove	0–0.1	5.26	0.28	0.022	19	244	116	15	352	111
	0.1–0.2	3.35	0.18	0.016	19	213	118	16	415	131
	0.2–0.3	3.06	0.17	0.014	18	224	107	14	247	91
	0.3–0.4	2.68	0.14	0.013	19	203	98	13	187	71
	0.4–0.5	2.11	0.10	0.015	21	138	98	13	246	106

P_o organic phosphorus, sum of $NaHCO_3$ P_o, NaOH P_o and residual *P* Ox - oxalate extraction; *Pyr* pyrophosphate extraction

Appendix 2

Microbial parameters, obtained from growth kinetics measured in microbial bioassays with samples of the six South African soils.

Site	Depth	BR	Max resp.		Slope		Lag time		tmax	
	(m)		C + N	C + N + P	C + N	C + N + P	C + N	C + N + P	C + N	C + N + P
		mg CO_2 h^{-1} g OM^{-1}			(h^{-1})		(h)			
Gauge Farm	0–0.1	0.123	4.7	10.9	0.007	0.051	16	18	223	68
	0.1–0.2	0.042	2.7	7.2	0.003	0.021	51	19	332	141
	0.2–0.3	0.093	2.8	5.7	0.011	0.022	32	31	237	212
	0.8–0.9	0.155	4.3	13.6	0.007	0.019	65	16	275	108
	1.2–1.3	0.052	4.4	12.6	0.005	0.021	151	65	615	179
	1.3–1.4	0.130	4.0	17.2	0.002	0.028	88	47	549	94
Salpine	0–0.1	0.057	3.6	7.5	0.005	0.046	19	8	267	74
	0.1–0.2	0.153	2.0	6.5	0.003	0.034	19	6	308	130
	0.2–0.3	0.065	1.4	5.4	0.002	0.050	20	15	501	139
	0.5–0.6	0.046	1.9	6.9	0.008	0.031	112	41	333	174
	0.7–0.8	0.022	1.6	6.1	0.007	0.018	152	64	701	197

(continued)

Site	Depth	BR	Max resp.		Slope		Lag time		tmax	
	(m)		C + N	C + N + P	C + N	C + N + P	C + N	C + N + P	C + N	C + N + P
		mg CO_2 h^{-1} g OM^{-1}			(h^{-1})		(h)			
Karkloof	0–0.1	0.045	1.9	5.5	0.008	0.058	36	30	283	81
	0.1–0.2	0.033	1.9	3.5	0.017	0.042	84	49	287	136
	0.2–0.3	0.022	1.3	2.5	0.004	0.029	160	59	602	259
	0.3–0.4	0.022	1.0	2.3	0.002	0.010	181	51	909	218
	0.4–0.5	0.014	1.2	3.4	0.003	0.013	236	42	1162	215
Westfield	0–0.1	0.037	3.7	6.7	0.026	0.043	40	31	164	105
	0.1–0.2	0.053	4.0	5.3	0.019	0.021	42	29	195	132
	0.2–0.3	0.044	1.1	2.1	0.007	0.020	21	22	626	220
	0.4–0.5	0.016	1.3	1.5	0.002	0.016	210	31	741	266
	0.5–0.6	0.003	0.1	0.3	0.007	0.003	690	39	2508	506
Pinewoods	0–0.1	0.037	2.7	7.0	0.014	0.050	27	15	254	100
	0.1–0.2	0.016	2.1	3.9	0.007	0.023	41	30	312	132
	0.2–0.3	0.007	2.0	2.9	0.005	0.023	66	42	355	189
	0.3–0.4	0.017	1.7	2.8	0.004	0.014	59	36	451	164
	0.4–0.5	0.003	1.6	2.5	0.004	0.014	105	28	574	233
Honey Grove	0–0.1	0.009	1.9	4.1	0.026	0.034	54	33	287	163
	0.1–0.2	0.027	2.5	3.4	0.017	0.025	58	21	201	128
	0.2–0.3	0.020	2.2	3.0	0.021	0.037	42	36	190	122
	0.3–0.4	0.020	1.6	2.8	0.009	0.034	75	86	351	191
	0.4–0.5	0.018	1.3	2.7	0.008	0.016	154	42	451	191

OM organic matter; *BR* basal respiration; *Max resp* maximum respiration rate; *Slope* the slope of the logarithmically transformed exponential growth phase; *Lag time* time from nutrient addition to start of exponential growth phase; *tmax* time to reach max resp; *C + N* addition of glucose and nitrogen; *C + N + P* addition of glucose, nitrogen and phosphorus

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