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Biochar increases soil enzyme activities in two contrasting pastoral soils under different grazing management

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ABSTRACT

Context. Soil enzyme activities are key regulators of carbon and nutrient cycling in grazed pastures. **Aims.** We investigated the effect of biochar addition on the activity of seven enzymes involved in the carbon, nitrogen and phosphorus cycles in a Sil-andic Andosol and a Dystric Cambisol under permanent pastures. **Methods.** The study consisted of a one-year field-based mesocosm experiment involving four pastures under different nutrient and livestock practices: with and without effluent under dairy cow grazing on the Andosol, and with either nil or high phosphorus fertiliser input under sheep grazing on the Cambisol. Soil treatments were: (1) willow biochar added at 1% w/w; (2) lime added at the liming equivalence of biochar (positive control); (3) no amendments (negative control). **Key results.** Compared with the Cambisol, the Andosol had higher dehydrogenase, urease, alkaline and acid phosphatase and, especially, nitrate-reductase activities, aligning with its higher pH and fertility. In both soils, biochar addition increased the activity of all enzymes, except for acid phosphatase and peroxidase; lime addition increased peroxidase and nitrate-reductase activity. **Conclusions.** The increased enzyme activity was strongly positively correlated with soil biological activity following biochar addition. Biochar caused a 40–45% increase in cellulase activity, attributed to increased root biomass following biochar addition. The response in acid and alkaline phosphatase activity can be attributed to the impact of biochar and lime addition on soil pH. **Implications.** The results provide more insights in realising the potential benefits of biochar to the provision of ecosystem services for grazed pastures.

Keywords: Andosol, biochar, biological activity, Cambisol, fertility, nutrient cycling, pasture, soil enzymes.

Introduction

A good understanding of how to best manage soils is important in order to maintain or increase the soil capability to meet human needs (Dominati *et al.* 2010). Soil organic matter (SOM) content and soil fertility play a key role in the ability of soils to be suitable for food production and provision of other ecosystem services (Adhikari and Hartemink 2016). Soil enzymes are an important part of soil processes, providing a link between soil biotic and abiotic components that are integral in nutrient and energy exchange within the soil (Yang and Wang 2002; Sinsabaugh *et al.* 2008). A measure of soil enzyme presence and activity allows for the indirect quantification of soil processes (flows) that contribute to carbon (C) and nutrient cycling, which in turn contribute to plant growth (Shi 2011; Jog *et al.* 2012), and soil detoxification and remediation (Rao *et al.* 2010). Soil enzymes are involved in multiple processes (Table 1), including the C, nitrogen (N), and phosphorus (P) cycles (Sardans *et al.* 2008; Das and Varma 2011). As a product of biological activity, enzymes are closely linked to abundance, community structure and activity of soil microorganisms, and soil micro- and meso-fauna (Caldwell 2005). Large soil animals, such as earthworms and some arthropods, influence the concentration and activity of soil enzymes in three ways: by releasing their own gut enzymes; by changing the microbial community inside their intestine and in their excreta; and by changing physico-chemical properties of the soil through their

Table 1. Sources and functions of selected soil enzymes.

Enzyme	Source	Soil function	Process	Factors influencing enzyme activity
Cellulase	Fungi, bacteria, protozoans	C-cycle	Decomposition of cellulose	Temperature, pH, water, quality, and location of soil organic matter (OM) (1)
Peroxidase	Fungi, bacteria, plants, invertebrates	C-cycle	Decomposition of lignin	Temperature, pH, soil aeration, soil OM content, management practices (2, 3)
Dehydrogenase	Bacteria and fungi	C-cycle	Oxidation of organic compounds	Soil water content and aeration, temperature, management practices (4)
Nitrate reductase	Bacteria, fungi, plant roots	N-cycle	Nitrate reduction to nitrite	Soil temperature, water content, pH (5, 6)
Urease	Bacteria, fungi, plants, some invertebrates	N-cycle	Hydrolysis of urea	Temperature, pH, soil OM content, management practices (3, 7)
Acid/alkaline phosphatase	Plants, fungi, bacteria	P-cycle	Hydrolysis of esters and anhydrides of phosphoric acid	Soil OM content, pH, management practices (8, 9)

Literature: (1) Deng and Tabatabai (1994*a*); (2) Sinsabaugh (2010); (3) Das and Varma (2011); (4) Wolińska and Stepniewska (2012); (5) Firestone (1982); (6) Abdelmagid and Tabatabai (1987); (7) Lloyd and Sheaffe (1973); (8) Eivazi and Tabatabai (1977); (9) Nannipieri *et al.* (2011).

burrowing and mixing activities (Moldenke *et al.* 2000; Kizilkaya *et al.* 2011). The dynamics of soil nutrient and biological properties and enzymatic activity help to identify the main drivers of the C, N and P biogeochemical cycles (Harrison 2016; Macdonald *et al.* 2018). Enzymes, therefore, can be seen as indicators that can be used to assess the influence of soil and plant management practices and land use on key soil ecosystem functions (Chang *et al.* 2007; Garbuz *et al.* 2016; Holík *et al.* 2019). The addition of organic amendments influences the physical and chemical environment of the soil, and affects the functional and structural diversity of soil microorganisms, which is the main factor for regulating and maintaining soil enzyme activity (Pérez-Piqueres *et al.* 2006; Cleveland *et al.* 2007). A promising organic amendment is biochar, a charcoal produced from biomass pyrolysis (Lorenz and Lal 2014; Lehmann *et al.* 2021). Biochar affects soil microbial communities through changes in soil bulk density, water retention, soil pH, and soil nutrient content and availability, as well as the provision of some labile C, especially when produced at low temperature of pyrolysis (Masto *et al.* 2013; De Tender *et al.* 2016). Generally, biochar application increases the abundance of soil microorganisms (Lehmann *et al.* 2011; Paz-Ferreiro *et al.* 2015; Palansooriya *et al.* 2019), whereas the influence of biochar on soil enzyme activity has been reported to be more variable, being highly dependent on the properties of the biochar and soil characteristics (Ouyang *et al.* 2014; Paz-Ferreiro *et al.* 2014; Khadem and Raiesi 2017; Garbuz *et al.* 2021).

Previous studies from our group (Garbuz *et al.* 2020, 2021) have shown that willow biochar addition (1% w/w ratio), when added to a Sil-andic Andosol and a Dystric Cambisol under grazed pasture, increased the size of both the bacterial and fungal communities, and also plant roots biomass within 12 months of application. In the same study, biochar also reduced soil bulk density (BD) and soil acidity, increased soil organic C and N stocks, and plant-available P (Olsen P). The changes in the stocks and availability of C, N and P indicate that biochar is also likely to be impacting on the activity of soil enzymes involved in the cycles of these three nutrients. However, it is not clear to what degree cellulase, peroxidase and dehydrogenase, which are involved in SOM decomposition, are affected by biochar. In addition, the degree of impact of biochar on nitrate-reductase and urease activity, which are involved in N cycling and used to quantify N transformation rates in soil, is unknown. It is well known that phosphatases, which are involved in mineralisation of organic P and, consequently, play a key role in plant P nutrition (Table 1), are sensitive to soil pH (Juma and Tabatabai 1978) and thus it is likely that their activity will be altered following biochar addition. In view of the above, we hypothesised that the influence of willow biochar produced at low temperature of pyrolysis on microbial biomass and plant root growth (1) should be mirrored in soil enzyme activity, as a part of underlying processes involved in stimulating the biogeochemical cycle regulating C, N and P supply, and (2) is beyond that of just the liming effect of biochar.

Materials and methods

Biochar production and characterisation

Biochar used in this experiment was produced from willow (*Salix matsudana* L.) chips. Air-dried feedstock (<12% moisture content) was pyrolysed at a maximum heating temperature of 350°C and residence time of 4 h. The characteristics of the biochar were: pH 7.8, organic C (C_{org}) 703 g kg^{-1}, the ratio of dichromate-oxidisable C out of C_{org} (C_{ox}/C_{org}) 51.4%, atomic H/C_{org} 0.63, liming equivalence 7.3% $CaCO_3$-eq (Garbuz *et al.* 2021). The biochar used in our study was classified as having a C storage class of 2, a liming class of 1, and a fertiliser class of zero (no nutrient value) (Camps-Arbestain *et al.* 2015). The choice of willow was based on the fact that it is readily available as it is used extensively in New Zealand in soil conservation and stream bank protection. Willow grows readily from a cutting and has an extensive rooting system.

Study sites

Two soils common under pastures in lower North Island of New Zealand were used in this study: (1) a Dystric Cambisol (IUSS Working Group WRB 2015), Brown soil in the New Zealand soil classification system (Hewitt 2010), from the experimental site of AgResearch Ballantrae Hill Country Research Station, Manawatu (40°18′35″S, 175°49′41″E); (2) a Sil-andic Andosol (IUSS Working Group WRB 2015), Allophanic soil (Hewitt 2010), from Hawera, Taranaki (39°36′28″S, 174°16′30″E). The Andosol used in this study is derived from volcanic ash. Rich in short-range order constituents, it offers high SOM protection, has a high anion retention, good physical properties, and resilience to treading pressure (Molloy 1998). The Cambisol is predominantly derived from loess materials. It has a low anion storage capacity and limited physical resilience to treading pressure. Two pastures grazed by dairy cows throughout the year on the Andosol were selected: one receiving dairy shed effluent (And-EF), and one not receiving effluent (And-NE). Both pastures receive 160 kg of N as fertiliser N ha^{-1} $year^{-1}$, 300 kg of 20% potash superphosphate ha^{-1} $year^{-1}$, and 1 kg selenium prill ha^{-1} $year^{-1}$ (a standard fertiliser regime for NZ pastoral soils under dairy farming). The two selected pastures on the Cambisol were grazed by sheep throughout the year: one (Cam-LF) had received no superphosphate since 1980, and the other (Cam-HF) receives 375 kg superphosphate ha^{-1} $year^{-1}$ since 1980 (Mackay *et al.* 2021).

Field-based mesocosm experiment

The field-based mesocosm experiment was conducted using large soil cores enclosed into PVC cylinders (150 mm Ø, 300 mm length). Four holes (5.1 cm Ø) were made in the wall of each cylinder to allow the free movement of soil organisms (fig. S1 in Garbuz *et al.* 2021). There were three treatments: (1) no amendments (negative control), (2) 1% of biochar application by weight (equivalent to approximately 10.9 Mg ha^{-1}), and (3) lime (positive control) applied at a rate corresponding to the liming equivalent of biochar (ca. 0.8 Mg ha^{-1}). Each treatment was replicated six times in each of the four pastures. During the southern hemisphere spring of 2017, the PVC cylinders were hammered into the ground and excavated with soil from each of the four pastures. At the laboratory, the turf layer (ca. 20 mm) was split off, and the top 150 mm of soil below the turf layer was removed from all cores. All earthworms from the topsoil were removed, counted, labelled with the core code, and cold-stored. For biochar and lime treatments, the soil was mixed with either biochar or lime, respectively, and put back into cylinders to the depth 20–170 mm; in control mesocosms the soil was also removed, and mixed without amendments. Earthworms and turf layer were placed back, and mesocosms installed in the field. Further details on design, preparation, and installation of mesocosms are provided in Garbuz *et al.* (2021). The experiment started in late October–November 2017, during the southern hemisphere spring. The sampling (18 cores from each pasture) occurred in November 2018, approximately 12 months after the start of the experiment. Climatological data for the two locations are provided in Supplementary Fig. S1.

Soil physico-chemical and biological properties

Soil samples for chemical analysis and microbial biomass were collected with a corer (30 mm Ø) from five depths: 0–20 mm (the turf), 20–95 mm, 95–170 mm, 170–200 mm, and 200–300 mm, and air-dried. The following variables were measured: soil bulk density (BD), pH, total C, total N (TN), nitrate-N (NO_3^-–N) and ammonium-N (NH_4^+–N), Olsen P. Inorganic C was negligible (<0.05%) in the lime-treated soil after 12 months of incubation, and thus total soil C was all organic (C_{org}). Mesofauna abundance (Collembola, Oribatida and Gamasina) was sampled by taking 50 mm × 50 mm × 50 mm cores from the topsoil (20–95 mm) in each mesocosm cylinder. Fungal and bacterial biomass were measured in mixed topsoil (20–170 mm) using the substrate-induced respiration (SIR) method with selective inhibition (Nakamoto and Wakahara 2004). Fungal (C_f) and bacterial (C_b) biomass C was calculated according to Anderson and Domsch (1978). The sum of C_f and C_b was considered as the microbial biomass. Earthworms from each mesocosm cylinder (full depth) were hand sorted, identified to species when possible, and counted. Plant roots from each cylinder were collected by hand, washed over a 3-mm sieve, oven-dried (40°C) and weighed. Soil properties are summarised in Garbuz *et al.* (2021) and we relate them to enzyme activities in this study.

Soil enzymes analysis

Soil samples for enzyme analysis were collected with a corer (30 mm Ø) from the same five depths as soil chemistry and microbial biomass samples. All soils were sieved (<2 mm) and air-dried prior to analysis. Alkaline and acid phosphatases, nitrate-reductase, urease, cellulase, peroxidase and dehydrogenase activity were measured in each of the five depths in all three treatments of the two contrasting soils. Details on soil enzyme analysis are provided in Garbuz *et al.* (2020) and in the supplementary information.

Statistical analysis

Statistical analysis was carried out using SAS 9.4. Normality of data sets was evaluated by the Shapiro–Wilk test. A multicollinearity analysis was done to check simple correlations and variance inflation factors for variables. The data were normalised using z-score prior to analysis. Analysis of variance (ANOVA) with contrast statements and Tukey HSD test were used to investigate the effect of factors: pasture (And-NE, And-EF, Cam-LF, Cam-HF), treatment (control, biochar, and lime) and pasture × treatment interaction on enzyme activities. When the interaction term was not significant, main effects were reported; if the interaction effect was significant, the four pastures were considered separately. Finally, for biochar-treated and control mesocosms we constructed a hypothetical model of causal relationships underlying the observed patterns in soil enzyme activity, biota, and nutrients, and used path analysis (proc CALIS in SAS 9.4) to calculate coefficients associated with each path in the model. Due to sample size, the hypothetical model of causal relationships underlying the observed patterns in soil C-enzymes activity was limited to cellulase and dehydrogenase.

Results

Results for the two soils, the Andosol and the Cambisol, are reported separately (Tables 2 and 3, respectively) unless otherwise indicated, as soil order had a significant effect on the activity of almost all enzymes. Phosphatases, urease, dehydrogenase and, especially, nitrate-reductase activities were higher in the Andosol, whereas cellulase activity was higher in the Cambisol (all $P < 0.005$). There was no difference in peroxidase activity between the two soil orders. In both soils, with few exceptions (e.g. peroxidase), enzyme activities declined with depth (all $P < 0.005$); the treatment effects on enzyme activity were observed primarily within the 20–95 mm and 95–170 mm soil depths, where treatments were applied, while in the turf and in layers below 170 mm there was no significant effect of treatments on soil enzyme activities (data not shown).

There were significant effects of pasture management practices on enzyme activities. Pastures with effluent addition (And-EF) and high P fertiliser input (Cam-HF) had higher phosphatases, nitrate-reductase and dehydrogenase activities (all $P < 0.005$) than their lower fertility equivalents (Tables 2 and 3, respectively).

Enzymes of the C cycle

Cellulase and dehydrogenase activities were higher in the biochar-treated soil than in the control (all $P < 0.001$), while the effect on peroxidase was site-dependent (Tables 2 and 3). In all pastures treated with lime, the activity of peroxidase was increased over that of the control and biochar-treated soil (all $P < 0.001$). Lime had no effect on cellulase activity, while its effect on dehydrogenase was site-dependent (Tables 2 and 3).

Path analysis (biochar-treated and negative control mesocosms only) identified significant pathways for cellulase and dehydrogenase (shown as C-enzymes) that offer a

Table 2. Activities of soil enzymes in experimental treatments of Andosol.

	Depth (mm)	And-NE			And-EF		
		Control	Biochar	Lime	Control	Biochar	Lime
Cellulase (mg glucose g^{-1} 24 h^{-1})	20–95	3.3b	4.9a	3.1b	3.9b	5.2a	4.1b
	95–170	2.1b	3.2a	2.1b	2.6b	3.4a	2.3b
Peroxidase (μmol *p*-benzoquinone g^{-1} h^{-1})	20–95	45.3b	47.3b	66.1a	28.3b	29.5b	45.0a
	95–170	38.5b	39.2b	64.1a	32.2c	27.8b	44.5a
Dehydrogenase (μg TPF g^{-1} 24 h^{-1})	20–95	1.7b	2.1a	2.1a	2.6b	2.9a	1.9c
	95–170	1.2b	1.6a	1.2b	1.7b	2.2a	1.3c
Nitrate reductase (μg NO_2^-–N g^{-1} 24 h^{-1})	20–95	28.4c	32.4b	37.0a	82.8c	85.2b	87.9a
	95–170	20.0b	21.1b	25.0a	41.8b	45.7b	60.1a
Urease (mg NH_4^+–N g^{-1} 4 h^{-1})	20–95	67.3b	70.2a	70.7a	100.0a	103.6a	101.1a
	95–170	54.2b	59.1a	58.7a	67.3a	69.9a	70.4a
Alkaline phosphatase (μg 4-nitrophenol g^{-1} h^{-1})	20–95	143.4b	157.2a	150.3b	198.6b	204.0a	209.7a
	95–170	90.8b	103.5a	96.4b	231.2c	240.1b	254.8a
Acid phosphatase (μg 4-nitrophenol g^{-1} h^{-1})	20–95	374.1a	351.4b	330.6c	460.8a	442.1b	435.4b
	95–170	278.3a	256.2b	236.6c	369.4a	335.9b	325.9b

Values represent means. Lowercase letters indicate significant differences (Tukey HSD test, $\alpha = 0.05$) between the treatments within a specific management. And-NE, Andosol, no effluent; And-EF, Andosol, effluent input; TPF, triphenylformazan.

Table 3. Activities of soil enzymes in experimental treatments of pastures on Cambisol.

	Depth (mm)	Cam-LF			Cam-HF		
		Control	Biochar	Lime	Control	Biochar	Lime
Cellulase (mg glucose g^{-1} 24 h^{-1})	20–95	7.3b	10.2a	7.3b	4.1b	6.2a	4.1b
	95–170	5.0b	8.7a	4.8b	3.1b	5.0a	3.4b
Peroxidase (μmol *p*-benzoquinone g^{-1} h^{-1})	20–95	17.8b	19.9b	43.4a	69.6c	60.5b	93.7a
	95–170	20.0b	21.9b	37.5a	61.5b	60.1b	83.7a
Dehydrogenase (μg TPF g^{-1} 24 h^{-1})	20–95	1.5b	2.0a	1.6b	1.6c	2.3a	2.0b
	95–170	1.0b	1.5a	1.3ab	1.0c	2.0a	1.7b
Nitrate reductase (μg NO_2–N g^{-1} 24 h^{-1})	20–95	1.8c	2.0b	2.8a	3.8b	4.2ab	4.0a
	95–170	1.5c	1.8b	2.7a	2.6b	2.9a	2.9a
Urease (mg NH_4^+–N g^{-1} 4 h^{-1})	20–95	32.7b	34.9a	33.2b	27.0b	29.5a	27.5b
	95–170	17.1b	20.0a	17.7b	18.2b	20.3a	17.9b
Alkaline phosphatase (μg 4-nitrophenol g^{-1} h^{-1})	20–95	94.2b	103.7ab	106.7a	105.2b	121.1a	120.0a
	95–170	59.9b	70.0b	73.0a	58.2b	70.1a	67.0a
Acid phosphatase (μg 4-nitrophenol g^{-1} h^{-1})	20–95	113.8a	94.6b	92.0b	160.1a	128.1b	119.9b
	95–170	79.8a	60.1b	52.6c	107.1a	90.8b	72.9c

Values represent means. Lowercase letters indicate significant differences (Tukey HSD test, $\alpha = 0.05$) between the treatments within a specific management. Cam-LF, Cambisol, low fertility; Cam-HF, Cambisol, high fertility; TPF, triphenylformazan.

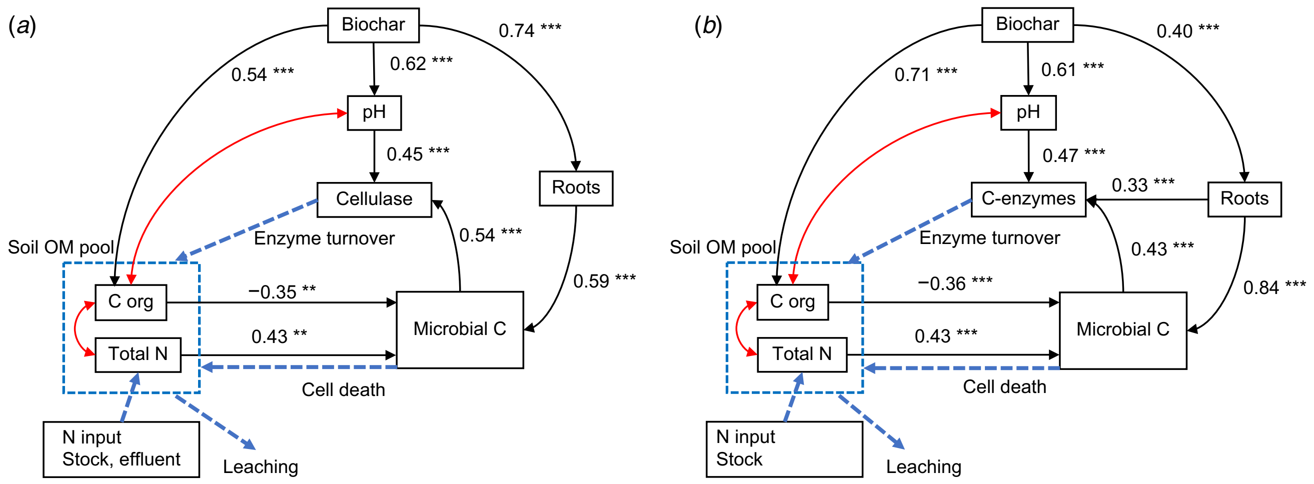

Fig. 1. Pathways for cellulase and dehydrogenase: (*a*) cellulase only in the Andosol pastures, (*b*) cellulase + dehydrogenase (C-enzymes) in the Cambisol pastures. Pathways for dehydrogenase in Andosol were not significant. Double-sided (red) arrows are correlations; dashed (blue) arrows represent pathways which were not quantified in the model. Both models significant, $P < 0.001$.

plausible representation of causal relationships (Fig. 1). In both soil types, the activity of cellulase (and C-enzymes for the Cambisol) was closely correlated with microbial biomass, with biochar addition acting as a significant driver for this variable, through changes in soil pH and the SOM pool (Fig. 1). The pathway involving roots in cellulase and dehydrogenase activity (C-enzymes) was important only in Cambisol pastures. In both soil types, the main source of C used by microbes appears to be root-derived, as opposed to the source of N, which appears to be derived from the soil pool, as described below. Pathways involving earthworms and mesofauna were not significant for cellulase and dehydrogenase, suggesting that these enzymes' activity is largely microbial-driven. On the other hand, pathways for peroxidase indicated the singular importance of pH for activity of this enzyme, with other factors not being significant (data not shown).

Enzymes of the N and P cycle

In both Cambisol pastures urease was higher in the biochar-treated soil than in the control or lime. In the Andosol, the soil receiving effluents (And-EF) had higher urease (all $P < 0.005$) than the same soil without effluent (And-NE), but no effect of biochar was detected. The activity of nitrate reductase was highest in soil treated with lime in both Andosol pastures, but also higher in biochar-treated soil than in control (Tables 2 and 3). Higher nitrate-reductase activity was correlated with higher earthworm abundances in sites with lower fertility (And-NE and Cam-LF) (both $P < 0.001$); data on earthworm abundance is provided in Garbuz *et al.* (2021). Path analysis suggests that the main drivers for nitrate-reductase activity in both the Andosol and Cambisol pastures were available N (NO_3^-–N) and soil pH (Fig. 2). Pathways involving soil fauna (earthworms and mesofauna) were not significant for nitrate reductase in either soil type. Pathways involving plant roots and microbial biomass in nitrate-reductase activity were important only in Cambisol pastures. Alkaline phosphatase activity was higher (and acid phosphatase lower) in the biochar- and lime-treated soils compared to the control (both $P < 0.005$).

Discussion

The increase in enzyme activity found in the present study was strongly correlated with greater soil biological activity and plant root biomass in the soils to which biochar had been added. The recurring question for all soil enzyme studies is whether enzyme production is constitutive (linked to the biomass of microbial cells) or inducible (linked to the presence of the substrate for the enzyme) (Moorhead *et al.* 2012, 2013). The theory of eco-enzymatic stoichiometry suggests that the relationships between microorganisms, enzymes and resources are tightly constrained (Sinsabaugh and Follstad Shah 2012). Further, a review of patterns between microbial biomass and specific enzyme activities reveals that the enzyme production is inducible and responsive to differences in substrate characteristics, and that patterns in C, N, and P acquisition are similar across soil types (Berg 2000; Allison 2005; Moorhead *et al.* 2013). In our study the enzyme production appears to be inducible, as increased activity of cellulase, peroxidase, dehydrogenase, nitrate reductase, urease, and alkaline phosphatase following biochar addition aligns with increased availability in soil C, N and P measured in the biochar-amended soil (Garbuz *et al.* 2021).

A number of studies have reported on various effects of biochar on soil biota and soil processes (Jones *et al.* 2012), and how those then influence the dynamics of soil biogeochemical cycles (Sarathchandra *et al.* 1988; Teutscherova *et al.* 2018; Holík *et al.* 2019). Further, different types of biochar influence bacterial and fungal activities differently, including shifts in the microbial community structure, as for example, changing fungi:bacteria ratio or changing abundance of specific groups of soil bacteria, all of which are responsible for increasing the diversity of the enzymatic pool in soil (Pandian *et al.* 2016; Gao *et al.* 2017; Garbuz *et al.* 2021). Most often, the addition of biochar causes an increase in enzyme activities (Vázquez *et al.* 2000; Paz-Ferreiro *et al.* 2014; Mierzwa-Hersztek *et al.* 2019). Wang *et al.* (2015*a*) showed that a small application rate of maize biochar produced at 450°C (0.5% w/w) increased the activity of enzymes involved in the C cycle, while larger application rates (>0.5%) had a negative effect on the activities of these enzymes. These authors also showed that enzymes

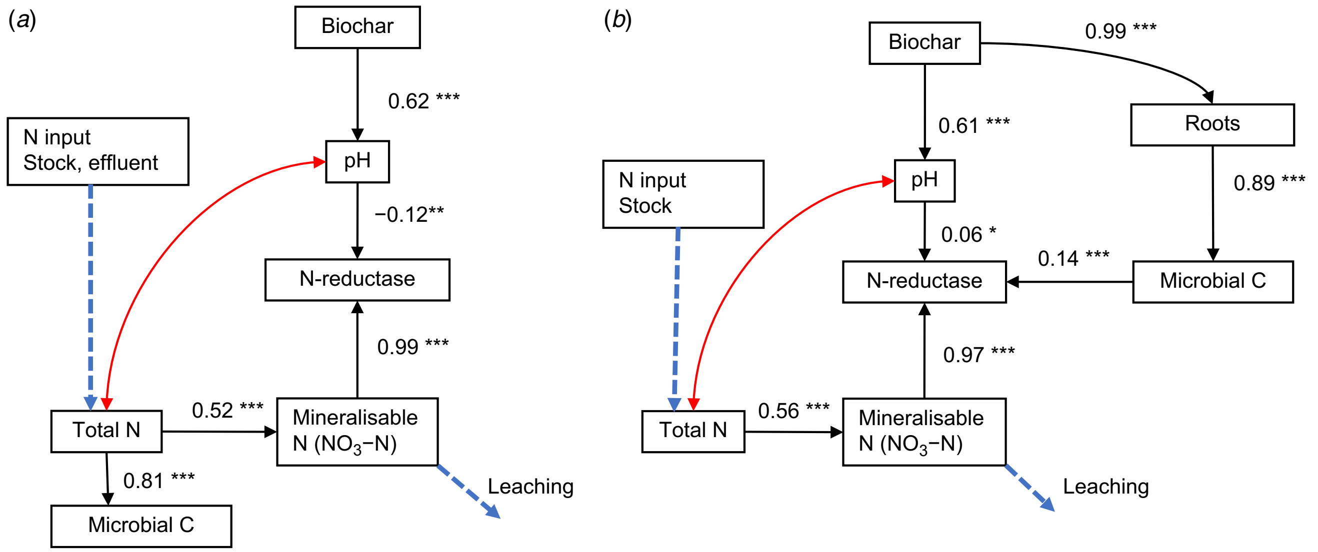

Fig. 2. Pathways for nitrate reductase in the (*a*) Andosol and (*b*) Cambisol pastures. Pathways for fauna (earthworms and mesofauna) were not significant. Double-sided (red) arrows are correlations; dashed (blue) arrows represent pathways which were not quantified in the model. Both models significant, $P < 0.001$.

involved in the N cycle increase with biochar application rate. The effect of biochar on selected enzymes appears to depend on soil chemical properties, available nutrients and SOM, as well as the properties of the biochar (Paz-Ferreiro *et al.* 2014; Irfan *et al.* 2019; Oladele 2019), such as its pH (in our biochar 7.8), C_{ox}/C_{org} (51.4%), and residual nutrient content (negligible).

Enzymes of the C cycle

The positive effect of biochar on dehydrogenase activity was attributed to an increase in microbial biomass C in biochar-treated soils (data for microbial biomass provided by Garbuz *et al.* 2021). Root C inputs to the rhizosphere, in response to higher root biomass, have been shown to stimulate soil enzyme activity and increase microbial biomass (Brzostek *et al.* 2013). Dehydrogenase is an important component of microbial metabolic functions (Casida 1977) and is often used as an indicator of soil microbial response to land use practice changes (Watts *et al.* 2010; Järvan *et al.* 2014). Biochar application can have a positive effect on dehydrogenase activity and increased C mineralisation in the soil as it has been observed in the short term (positive priming) (Ouyang *et al.* 2014; Lehmann *et al.* 2021) which can be related, to some extent, to the presence of labile C supplied in biochar as well as its labile C to labile N ratio if residual N is present (Wang *et al.* 2012). The high C_{ox}/C_{org} ratio and the relatively high atomic H/C_{org} ratio suggests the presence of a considerable fraction of labile C in our biochar. This probably favoured microbial growth and positive priming of native SOM as inferred from a mass balance calculation (Garbuz *et al.* 2021). However, over one year period, biochar-treated soils in our study showed a 1.2 to 4.0 Mg C ha^{-1} gain in root C, especially high in Cambisol pastures, which may generate a negative priming over time (Garbuz *et al.* 2021). Biochar can promote long-term C storage through stabilisation of rhizo-deposits and organic ligands, in general, on biochar surface, as it can act as a new reactive surface (Lehmann *et al.* 2021).

The increased cellulase activity in biochar-treated soils was correlated with increased root biomass, suggesting that the increased supply of root necromass provided additional substrate for cellulase activity (Sajjad *et al.* 2002; Sinsabaugh 2010), yet the path analysis suggests that, in the Andosols, this only occurs indirectly through the impact of the enhanced root growth on microbial biomass. The amount of cellulose in our biochar is negligible given that the biochar was produced at 350°C and cellulose is fully carbonised at temperatures above 240°C (Demirbaş 2001). The path analysis indicated both similarities and differences in the drivers of C-enzyme activities in the Andosol and Cambisol pastures. The similarities reflect that, as expected, the most important driver is soil biological activity, while the differences are likely to be a manifestation of different types of SOM, soil aggregation and microbial community structure between the two soils. For example, the fact that in the Cambisol, but not in the Andosol, roots have a direct impact on both measured enzymes involved in the C cycle, could be related to the existence of a more plant-derived OM in the Cambisol, and a more microbial-derived SOM in the Andosol (Herath *et al.* 2015) due to differences in clay mineralogy and prevalence of microaggregates in the latter (Angst *et al.* 2021). The increase in root biomass reported by Garbuz *et al.* (2021) would have caused a direct increase in microbial-derived SOM in both soil types, but this increase would have been diluted in the Andosol where this SOM fraction is already more abundant as suggested by Wang *et al.* (2015*b*).

Peroxidase activity generally increases with soil pH (Sinsabaugh 2010). With an increase in pH, the bonds of organic molecules (ligands) with mineral surfaces become weaker, and desorbed SOM becomes more easily degraded and oxidised by peroxidases (Sinsabaugh 2010; Tian and Shi 2014).

Enzymes of the N cycle

Nitrate reductase activity is affected by factors such as nitrate concentration and soil pH, with an optimum at pH 7 (Abdelmagid and Tabatabai 1987). This is consistent with the enzyme activity trends observed in this study: (1) nitrate-reductase activity was highest in the Andosol that received effluents (And-EF) and in the Cambisol with high fertility pasture containing an active legume component (Cam-HF); (2) both lime and biochar increased nitrate-reductase activity, with lime having a more pronounced effect. Andosols are abundant in micropores and microaggregates which can remain saturated with water for long periods under udic moisture conditions (Buurman *et al.* 2007), as experienced during the study, thus creating favourable anaerobic conditions for nitrate reduction. This, and high substrate availability, could explain the higher nitrate-reductase activity in this soil (Abdelmagid and Tabatabai 1987).

Substrate availability (NO_3^-–N) in biochar-treated topsoil in our experiment was higher or the same as in control and lime treatments (Garbuz *et al.* 2021) but, compared to lime, biochar mesocosms had a lower nitrate-reductase activity. In our study, biochar-treated soil had significantly lower bulk density and higher root biomass (Garbuz *et al.* 2021); we speculate that this would be associated with increased soil aeration, thereby limiting the activity of nitrate reductase (Abdelmagid and Tabatabai 1987; Joseph *et al.* 2015). Moreover, it is well known that biochar has an effect on redox-regulated N transformations (Chacón *et al.* 2017). In fact, when produced at low temperature, such as the one used in this study, biochar can act as an electron shuttle (Chacón *et al.* 2017; Dai *et al.* 2021), favouring the full reduction of NO_3^- to N_2 (Obia *et al.* 2015). Yet the impact of biochar electrochemical properties on N-reductase activity is hard to discern with the data available.

There is evidence that biochar and lime affect different groups of soil bacteria responsible for denitrification (Bai *et al.* 2015; Jha *et al.* 2016; Harter *et al.* 2017; Weldon *et al.* 2019). The positive correlation between earthworm abundance and the activity of nitrate reductase in two of the pastures supports the idea of a link between earthworms and denitrifying bacteria, suggested by some authors (Burtelow *et al.* 1998; Depkat-Jakob *et al.* 2010) through increased nitrate input by earthworms. Garbuz *et al.* (2020), working with the same soils under glasshouse conditions, also showed synergetic interactions between lime, earthworms, and increased nitrate-reductase activity.

The high urease activity in the Andosol might reflect the higher urine input from the lactating dairy cows, up to 55 L urine cow^{-1} day^{-1} (Betteridge *et al.* 1986), compared to 3 L urine sheep^{-1} day^{-1} (Ledgard *et al.* 2008) from sheep grazing in Cambisol. Urease activity has been reported to be strongly correlated with soil bacterial biomass (Amini Kiasari *et al.* 2018). In our experiment bacterial biomass increased with the addition of biochar (Garbuz *et al.* 2021), and was correlated with urease activity in all pastures except And-NE. The latter may be explained by the fact that the bacteria–urease correlation relies on a specific group of ureolytic bacteria, but not on the whole bacterial community (Lloyd and Sheaffe 1973). In other studies, urease activity in response to biochar addition has also been unpredictable; for example, rice husk biochar had both negative and positive effects on urease activity in two different acid soils – Ultisol with a pH 5.8, C_{org} 16 g kg^{-1} and available N 1.6 g kg^{-1} and Alfisol with a pH 4.4, C_{org} 3.7 g kg^{-1}, and available N 0.0156 g kg^{-1} (Huang *et al.* 2017; Oladele 2019).

Enzymes of the P cycle

As expected, the addition of alkaline material (lime or biochar) drove an increase in the alkaline phosphotase to acid phosphotase (AlP/AcP) ratio, reflecting the sensitivity of phosphatases to soil pH (Acosta-Martínez and Tabatabai 2000), with activity of acid phosphatase decreasing and alkaline phosphatase increasing. Olsen P values in the soil amended with biochar were higher than in the control in both Andosols and in the Cambisol with high fertility (Garbuz *et al.* 2021), so the higher alkaline phosphatase activity in the biochar-treated soils compared with the control reflects increased substrate availability. However, this was not the case in the Cambisol with low fertility, indicating that despite an increase in the AlP/AcP ratio, this does not always translate into an increase in plant-available P. In addition to causing an increase in available P through desorption due to increased pH, biochar amendment may also influence P availability because of the increase in SOM. An enrichment in organic ligands would result in chelation of Al^{3+} and Fe^{3+} that would otherwise precipitate P (Gao and DeLuca 2016; Gao *et al.* 2019).

The higher phosphatase activity in the Andosol than Cambisol soil can be explained by the fact that the short-range order inorganic constituents (e.g. allophane) abundant in Andosols have the capacity to immobilise phosphatase (Chatterjee *et al.* 2014; Jordanova 2017) and protect this enzyme from adverse conditions (Shindo *et al.* 2002). Phosphatase activities are positively correlated to SOM, which enhances the stability of these enzymes (Bonmati *et al.* 1991). As root biomass was enhanced in the biochar treatment (Garbuz *et al.* 2021), we can hypothesise that root-derived SOM increased alkaline phosphatase activity.

Conclusions

Studying the effect that biochar addition has on enzyme activity, a key regulator of C and nutrient cycling of grazed pastures, is important to fully understand the potential benefits of biochar application to the provision of soil ecosystem services. Willow biochar pyrolysed at low temperature and applied at a rate of 10.9 Mg ha^{-1} had a significant influence on the activities of many of the enzymes involved in C, N and P cycling through a diversity of mechanisms. In our study, the enzyme production appears to be inducible, as increased activity of cellulase, peroxidase, dehydrogenase, nitrate reductase, urease and alkaline phosphatase following biochar addition aligns with increased availability in soil C, N and P measured in the biochar-amended soil. The effects of biochar range from (1) stimulating plant roots and/or the soil microbial community, which resulted in a parallel increase in cellulase and dehydrogenase activity, through to (2) increasing soil alkalinity and/or nutrient stocks, that favours, except for acid phosphatase, the activity of all enzymes. Interestingly, the increase in urease activity may point to the influence of biochar on specific functional groups within the wider soil biological community. Future research is required to better understand the influence biochar addition has on each of the functional groups that make up the soil biological community, and the flow-on effect that has on the enzymes involved in the C, N and P cycles.

Supplementary material

Supplementary material is available online.

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