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Enzymatic activities and microbial communities in an Antarctic dry valley soil: Responses to C and N supplementation

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ABSTRACT

The soils of the Antarctic dry valleys are exposed to extremely dry and cold conditions. Nevertheless, they contain small communities of micro-organisms that contribute to the biogeochemical transformations of the bioelements, albeit at slow rates. We have determined the dehydrogenase, β-glucosidase, acid and alkaline phosphatase and arylsulphatase activities and the rates of respiration (CO_2 production) in laboratory assays of soils collected from a field experiment in an Antarctic dry valley. The objective of the field experiment was to test the responses of the soil microbial community to additions of C and N in simple (glucose and NH_4Cl) and complex forms (glycine and lacustrine detritus from the adjacent lake comprising principally cyanobacterial necromass). The soil samples were taken 3 years after the experimental treatments had been applied. In unamended soil, all enzyme activities and respiration were detected indicating that the enzymatic capacity to mineralize organic C, P and S compounds existed in the soil, despite the very low organic matter content. Relative to the control (unamended soil), respiration was significantly increased by all the experimental additions of C and N except the smallest NH_4Cl addition (1 mg N g^{-1} soil) and the smallest detritus addition (1.5 mg C g^{-1} soil and 0.13 mg N g^{-1} soil). The activities of all enzymes except dehydrogenase were increased by C and combined large C (10 mg C g^{-1} soil) and N additions, but either unchanged or diminished by addition of either N only or N (up to 10 mg N g^{-1} soil) with only small C (1 mg C g^{-1} soil) additions in the form of glucose and NH_4Cl. This suggests that in the presence of a large amount of N, the C supply for enzyme biosynthesis was limited. When normalized with respect to soil respiration, only arylsulphatase per unit of respiration showed a significant increase with C and N additions as glucose and NH_4Cl, consistent with S limitation when C and N limitations have been alleviated. Based on the positive responses of enzyme activity, detritus appeared to provide either conditions or resources which led to a larger biological response than a similar amount of C and more N added in the form of defined compounds (glucose, NH_4Cl or glycine). Assessment of the soil microbial community by ester-linked fatty acid (ELFA) analysis provided no evidence of changes in the community structure as a result of the C and N supplementation treatments. Thus the respiration and enzyme activity responses to supplementation occurred in an apparently structurally stable or unresponsive microbial community.

1. Introduction

The Antarctic dry valleys occupy a region of Antarctica characterized by a combination of low temperatures and lack of liquid water that seriously limit the abundance and activity of terrestrial organisms. However, the soils in the Antarctic dry valleys contain organic C (Burkins et al., 2000; Barrett et al., 2005; Elberling et al., 2006), emit CO_2 produced by respiration (Burkins et al., 2002; Parsons et al., 2004; Barrett et al., 2006; Elberling et al., 2006; Hopkins et al., 2006b) and support communities of heterotrophic soil organisms (Friedmann, 1982; Treonis et al., 1999; Stevens and

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Hogg, 2002). There are several potential sources of organic C to support terrestrial foodwebs, including algae (Parker et al., 1982; Greenfield, 1998; Elberling et al., 2006; Hopkins et al., 2006b), marine detritus (Burkins et al., 2000), *in situ* primary production by mosses, lichens including endolithic lichens, terrestrial cyanobacteria and terrestrial algae (Friedmann, 1982; Friedmann et al., 1993; Friedmann and Ocampo, 1976; Green et al., 1998; Kappen et al., 1998; Pannewitz et al., 2005; Schwarz et al., 1992; Novis et al., 2007) and the remnants of ancient organic deposits from palaeolakes (Burkins et al., 2000). The organic matter contents of the soils are often greater close to the edges of lakes (Moorhead et al., 2003; Elberling et al., 2006; Hopkins et al., 2008) and these areas can be hot-spots of biological activity in the dry valleys (Elberling, et al., 2006; Gregorich et al., 2006; Hopkins et al., 2006a,b, 2008).

Soil enzyme activities can provide two types of information. They can act as an indicator of potential microbial activity and hence their activities often correlate moderately well with other indicators of activity such as soil respiration, ATP content and microbial biomass (Dick, 1997). Soil enzyme activities may also provide some insight into the metabolic repertoire of the soil so that the potential for transformation of specific sources of energy or nutrients can be assessed (Shaw and Burns, 2006) and thus indicate the relative availability or limitation of particular energy or nutrient sources in the environment. However, some caution is needed in the interpretation of soil enzyme assays because activities are usually assessed under optimized conditions and are the integrated activity of enzymes from living and dead organisms, plus stabilized extracellular enzymes (Burns, 1978).

We obtained soil samples from a field experiment in the Garwood Valley, Ross Dependency, Antarctica (78°01′S; 163°53′E; Fig. 1) set up to investigate the effects of supplementation with C and N on soil processes. The objectives of this study were to assess the activity of enzymatic functions relevant to the biogeochemical transformations of organic C, P and S in soil, and to test how these activities and the structure of the microbial community respond to organic C and both inorganic and organic N additions which simulate the re-distribution of lacustrine detritus on the soils.

2. Materials and methods

2.1. Experimental study site

The field experiment was set up in an area of frost-heave polygons in the Garwood Valley (Fig. 1). The experiment comprised 92 circular plots with 25 cm radius each marked in the centre with an aluminium stake. The plots were arranged in a randomized block design with four blocks each on a separate polygon (Fig. 1). The soil contained 1.1 mg organic C g^{-1}, 0.05 mg total N g^{-1}, 1.1 μg NO_3^--N g^{-1} and 1.0 μg NH_4^+-N, and had pH 8.4. It was composed mainly of sand-sized particles and contained a negligible quantity of clay-sized particles; more comprehensive site and soil details are provided by Elberling et al. (2006) and Hopkins et al. (2006b).

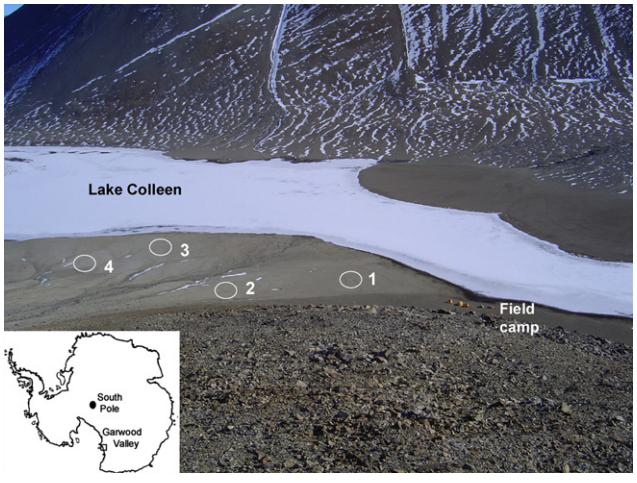

Fig. 1. The Garwood Valley viewed from the SSE in January 2005. The positions of the four experimental blocks are shown (1–4).

2.2. Experimental design

The experimental treatments were made over a period of 3 days during January 2003 and are described in Table 1. There were four, eight or 12 replicates of each treatment, i.e. depending on the treatments there were one, two or three replicates per block. The treatments were applied following a period of light snow (2–3 cm accumulation) which was removed from the surface of each plot before the treatments were applied. The experimental amendments were applied to the soil surface and mixed to a depth of 5 cm. The snow was replaced and it then either melted, sublimed or was ablated from the soil surface over the following 2–3 days. In the work described here, soil samples from all 92 plots were collected in January 2006. The soil samples from 0 to 5 cm depth were sieved to pass a 2 mm sieve in the field, sealed in two polythene bags and stored at the field temperature (typically in the range −5 to +2 °C) for up to 7 days before transport to Scott Base on Ross Island, where they were stored below 0 °C until transport to New Zealand and subsequent transport by refrigerated air freight to the UK. The samples were stored frozen in the UK until the start of analysis when sub-samples were warmed to 10 °C for 7 days before analysis.

2.3. Soil respiration

Soil samples (50 g fresh wt) were adjusted to 20% water-holding capacity by the addition of cooled (10 °C) distilled water and allowed to equilibrate at 10 °C for 24 h after water addition. The soils were then incubated for 7 days at 10 °C in the dark in sealed Nalgene bottles each containing a glass vial of 10 ml of 0.6 M KOH. The CO_2 produced from the soil was measured by back-titrating the excess KOH with 0.5 M HCl (Hopkins et al., 1988). The incubation temperature of 10 °C was used because this was representative of the highest soil temperatures recorded during the austral summer and to allow comparison with previous laboratory measurements for soil from the same site (Hopkins et al., 2006b).

2.4. Soil enzyme assays

The activities of β-glucosidase (EC 3.2.1.21), alkaline phosphatase (EC 3.1.3.21), acid phosphatase (EC 3.1.3.21) and arylsulphatase (EC 3.1.6.1) were assayed using the colorimetric determination of *p*-nitrophenol released when soil was incubated with *p*-nitrophenyl β-D-glucopyranoside in pH 6.5 buffer, *p*-nitrophenyl phosphate in pH 11 buffer, *p*-nitrophenyl phosphate in pH 6.5 buffer and *p*-nitrophenyl sulphate in pH 6.5 buffer, respectively (Tabatabai and Bremner, 1969; Tabatabai, 1994; Alef and Nannipieri, 1995). Briefly, 1 g samples of field-moist soil were weighed into 30 ml glass vials, 4 ml of buffer and 1 ml substrate were added, mixed thoroughly and incubated for 1 h at 37 °C after which time 1 ml of 0.5 M $CaCl_2$ and either 4 ml Tris buffer pH 12 for the β-glucosidase assay or 4 ml of 0.5 M NaOH for the phosphatase and arylsulphatase assays were added. The resulting suspensions were filtered immediately through Whatman 2 filter paper and the absorbance at 400 nm was measured using a spectrophotometer. Dehydrogenase activity was determined using colorimetric determination of iodonitrotetrazolium chloride (formazan, INF) formed when soil was incubated with 2(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT) (von Mersi and Schinner, 1991; Alef and

Table 1
Summary of experimental treatments applied to field plots in the Garwood Valley in January 2003

Treatment code (see Figs. 2–5)	Treatment description	Amendment	Number of replicates
0	Control	Nil	12
C1	Low glucose	1 mg C g^{-1} soil	8
C10	High glucose	10 mg C g^{-1} soil	8
N1	Low NH_4Cl	1 mg N g^{-1} soil	8
N10	High NH_4Cl	10 mg N g^{-1} soil	8
C1N1	Low glucose, low NH_4Cl	1 mg C g^{-1} soil 1 mg N g^{-1} soil	8
C1N10	Low glucose, high NH_4Cl	1 mg C g^{-1} soil 10 mg N g^{-1} soil	8
C10N1	High glucose, low NH_4Cl	10 mg C g^{-1} soil 1 mg N g^{-1} soil	8
C10N10	High glucose, high NH_4Cl	10 mg C g^{-1} soil 10 mg N g^{-1} soil	8
AA1	Low glycine (amino acid)	1.7 mg C g^{-1} soil 1 mg N g^{-1} soil	4
AA10	High glycine (amino acid)	17 mg C g^{-1} soil 10 mg N g^{-1} soil	4
Det1	Low lacustrine detritus	25 mg detritus g^{-1} soil supplying 1.5 mg C g^{-1} soil and 0.13 mg N g^{-1} soil	4
Det10	High lacustrine detritus	250 mg detritus g^{-1} soil supplying 15 mg C g^{-1} soil and 1.3 mg N g^{-1} soil	4

Nannipieri, 1995). Briefly, 1 g of moist soil was weighed into 30 ml glass vials, 1.5 ml of pH 7.0 Tris buffer and 2 ml of 10 mM INT solution were added, mixed thoroughly and incubated for 2 h at 37 °C after which 10 ml of a 50:50 mixture of *N*,*N*-dimethylformamide and ethanol was added to extract the INF. The suspensions were filtered through Whatman 2 filter paper and the absorbance at 464 nm was measured using a spectrophotometer. For all enzyme assays, autoclaved soil samples were used as controls.

2.5. Microbial lipid analyses

Microbial lipid analysis was used to assess whether the experimental treatments affected the microbial community structure. Ester-linked fatty acids (ELFAs) were extracted and analysed by gas chromatography (Frostegård et al., 1991; Schutter and Dick, 2000). Soil from the detritus treatments were omitted from the ELFA analysis to avoid the confounding effects of fatty acids from the detritus. ELFA analysis was used in preference to the more routinely employed phospholipid fatty analyses (PLFA) because ELFA analysis is more rapid and both PLFA and ELFA approaches are comparable in their ability to discriminate between microbial communities (Hinojosa et al., 2005; Griffiths et al., 2007).

2.6. Statistical analyses

The effects of amendment treatments on enzyme activities and enzyme activities per unit respiration were tested by analyses of variance, using S-Plus version 7 (Insightful Corp., 2005). Response data were either logarithm- or square root-transformed to meet assumptions of normality. For each enzyme, the effects of glucose and NH_4Cl additions were analyzed by a two-factor ANOVA, with each factor having three levels (0, 1 and 10). The effects of amino acid and detritus additions were analyzed by separate, single-factor ANOVAs each with three levels (0, 1 and 10).

Multivariate analysis on the complete set of enzyme activity data was conducted in S-Plus version 7 (Insightful Corp., 2005). All replicates of all 13 treatments were included and response data were logarithm- or square-root transformed to satisfy assumptions of normality. Separate analyses were undertaken for both enzyme activities and activities per unit respiration. Each of these data sets was subjected to both principal component analysis to assess the covariance structure of the activity data and linear discriminant analysis to identify weightings for enzyme responses according to ability to separate the thirteen treatment groups (Legendre and Legendre, 1998).

ELFA community profiles were analyzed using principal component analysis and the principal components were analyzed using ANOVA using Genstat (Lawes Agricultural Trust, VSN International, Hemel Hempstead, UK).

3. Results

3.1. Enzymatic activities and respiration in unamended soils

The activities of all five enzymes assayed and respiration were detected in the unamended (control) soil; they were all significantly greater than zero (Table 2). Although they cannot be related to actual process rates in the field, the enzyme activities do indicate the capacity of the soil to undertake biogeochemical transformations of organic C, P and S. Respiration was measured without added substrate and providing a more direct measure of metabolic activity in the soils.

3.2. Respiratory responses to experimental treatments

Respiration was significantly increased by all experimental treatments except the smallest NH_4Cl addition, and there were additive effects of C and N from both glucose plus NH_4Cl and glycine (Fig. 2). The addition of detritus also led to increased respiration (Fig. 2; Table 2) despite the smaller amounts of C and N in this material compared to the glucose, NH_4Cl and glycine treatments (Table 1).

3.3. Enzymatic responses to C and N supplementation

Relative to the control, dehydrogenase activity was reduced by all combinations of glucose and NH_4Cl, whereas the activities of the other four enzymes were only depressed by NH_4Cl additions in the presence of either low or no glucose (Fig. 3). When high glucose and either high or low NH_4Cl were added, the activities of β-glucosidase, the two phosphatases and arylsulphatase were significantly increased (Fig. 3). The addition of glycine had similar effects on the enzyme activities to the glucose plus NH_4Cl treatments for dehydrogenase (i.e. depression relative to the control), and β-glucosidase and arylsulphatase (i.e. increases relative to the control; which were marginal in the case of arylsulphatase) (Fig. 3). However, for the phosphatases, glycine led to either no effect or a depression in activity (Fig. 3), whereas C and N addition as glucose and NH_4Cl increased activity (Fig. 3). The addition of detritus led to significant increases in all enzyme activities relative to the control, other than dehydrogenase (Fig. 3).

Normalizing the enzyme activities with respect to respiration had no effect on the response of dehydrogenase activity to glucose and NH_4Cl additions (Fig. 4). Most of the positive effects of glucose and NH_4Cl additions on the activities of β-glucosidase and the phosphatases were associated with the increases in respiration because when they were normalized with respect to respiration, the enzyme activities were either depressed or showed no significant response relative to the control (Fig. 4). The positive effects of glucose and NH_4Cl addition on arylsulphatase activity were only maintained in two cases after normalizing for respiration (Fig. 4). The effects of glycine addition were similar to those of glucose plus NH_4Cl on dehydrogenase and the phosphatases (i.e. depressions relative to the control) after normalizing for respiration (Fig. 4). As

Table 2
Enzyme activities and respiration rates for soils from the Garwood Valley, Antarctica amended with different organic C and either inorganic or organic N sources, plus lacustrine detritus

Activity, units and (transformation used)	Statistical parameter	Treatment												
		Control (unamended)	Low glucose	High glucose	Low NH_4Cl	High NH_4Cl	Low glucose, low NH_4Cl	Low glucose, high NH_4Cl	High glucose, low NH_4Cl	High glucose, high NH_4Cl	Low glycine	High glycine	Low detritus	High detritus
Acid phosphatase μg C g^{-1} soil h^{-1} (logarithmic transformed)	Mean	0.064	0.108	0.125	0.051	0.035	0.081	0.053	0.240	0.129	0.079	0.061	0.095	0.117
	Lower 95% confidence interval	0.040	0.065	0.073	0.022	0.013	0.046	0.030	0.133	0.062	0.023	0.020	0.044	0.044
	Upper 95% confidence interval	0.102	0.180	0.214	0.119	0.096	0.145	0.094	0.432	0.267	0.273	0.182	0.208	0.315
Alkaline phosphatase μg C g^{-1} soil h^{-1} (logarithmic transformed)	Mean	0.413	0.582	0.495	0.332	0.148	0.474	0.127	0.861	0.465	0.409	0.256	0.505	0.604
	Lower 95% confidence interval	0.351	0.401	0.400	0.251	0.109	0.314	0.052	0.666	0.233	0.296	0.114	0.443	0.255
	Upper 95% confidence interval	0.486	0.845	0.614	0.439	0.201	0.714	0.312	1.113	0.928	0.565	0.575	0.577	1.433
Aryl sulphatase μg C g^{-1} soil h^{-1} (logarithmic transformed)	Mean	0.0098	0.0144	0.0537	0.0124	0.0130	0.0149	0.0170	0.0625	0.0385	0.0154	0.0125	0.0150	0.0133
	Lower 95% confidence interval	0.0078	0.0112	0.0313	0.0087	0.0096	0.0081	0.0123	0.0410	0.0197	0.0074	0.0073	0.0079	0.0098
	Upper 95% confidence interval	0.0124	0.0186	0.0919	0.0178	0.0176	0.0274	0.0235	0.0953	0.0751	0.0321	0.0213	0.0282	0.0182
β-Glucosidase μg C g^{-1} soil h^{-1} (logarithmic transformed)	Mean	0.0359	0.0571	0.0507	0.0365	0.0166	0.0461	0.0266	0.0679	0.0700	0.0445	0.0971	0.0502	0.0873
	Lower 95% confidence interval	0.0306	0.0442	0.0355	0.0224	0.0129	0.0317	0.0169	0.0455	0.0312	0.0157	0.0475	0.0296	0.0307
	Upper 95% confidence interval	0.0421	0.0739	0.0723	0.0596	0.0215	0.0672	0.0420	0.1014	0.1573	0.1259	0.1984	0.0849	0.2479
Dehydrogenase μg C g^{-1} soil h^{-1} (logarithmic transformed)	Mean	3.260	2.866	1.961	1.756	0.559	2.363	1.090	2.441	1.587	1.449	1.960	2.898	4.329
	Lower 95% confidence interval	2.438	2.161	1.346	1.114	0.192	1.580	0.747	1.595	0.755	0.361	0.820	1.281	2.527
	Upper 95% confidence interval	4.358	3.800	2.858	2.769	1.632	3.534	1.591	3.737	3.334	5.819	4.686	6.555	7.416
Respiration μg C g^{-1} soil day^{-1} (square root transformed)	Mean	1.828	4.801	16.132	2.072	3.356	6.826	4.927	19.923	25.011	3.013	7.102	5.236	7.298
	Lower 95% confidence interval	0.839	2.878	13.524	1.250	2.029	4.134	3.126	17.435	21.758	1.307	3.672	1.843	4.335
	Upper 95% confidence interval	3.197	7.214	18.970	3.100	5.014	10.191	7.136	22.578	28.490	5.420	11.653	10.361	11.029
	N	12	8	8	8	8	8	8	8	8	4	4	5	5

These data are back-calculated from data transformed by logarithmic or square root functions applied to ensure normal distributions before statistical analyses.

Fig. 2. Responses after 3 years of soil respiration to organic C and N amendments in the Garwood Valley, Antarctica. The values are the *P* values from analysis of variance with the +/− sign indicating the direction of the effect and the significance indicated as $^{*}0.20 > P > 0.05$, $^{**}0.05 > P > 0.01$, $^{***}0.01 > P > 0.001$, and NS, $P > 0.20$.

with the gross enzyme activities (Fig. 3), glycine led to increased activities of β-glucosidase normalized for respiration. The positive effects observed for detritus addition on persisted only for β-glucosidase after normalizing for respiration. In the case of dehydrogenase, the phosphatases and arylsulphatase, the effect of detritus on the respiration-normalized activity was not significant (Fig. 4).

Fig. 3. Responses after 3 years of soil enzymes activities to organic C and N amendments in the Garwood Valley, Antarctica. The values are the *P* values from analysis of variance with the +/− sign indicating the direction of the effect and the significance indicated as $^{*}0.20 > P > 0.05$, $^{**}0.05 > P > 0.01$, $^{***}0.01 > P > 0.001$, and NS, $P > 0.20$.

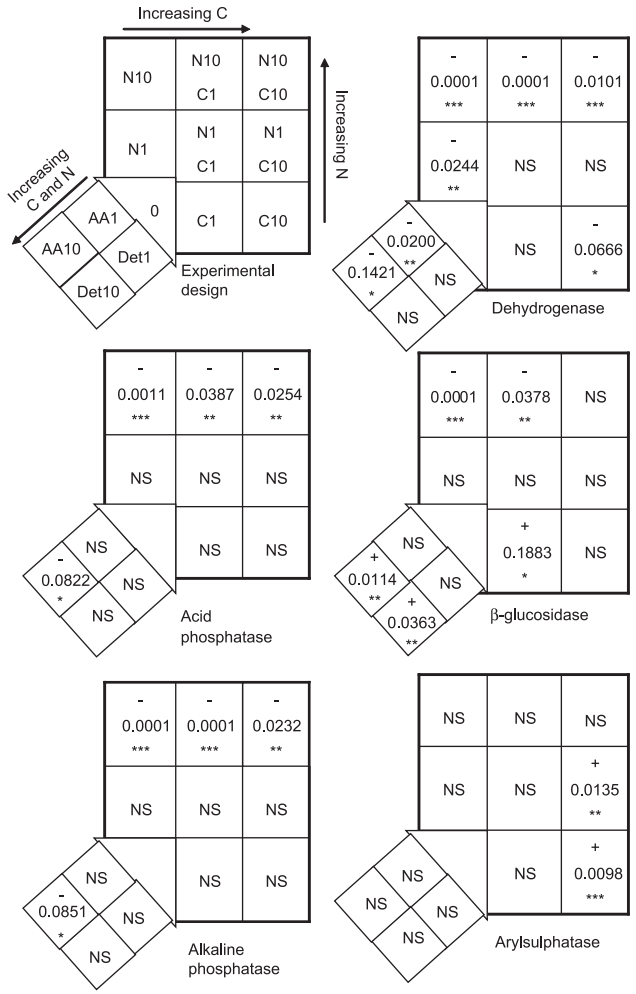

Fig. 4. Responses after 3 years of soil enzymes activities normalized for differences in respiration rate to organic C and N amendments in the Garwood Valley, Antarctica. The values are the *P* values from analysis of variance with the +/− sign indicating the direction of the effect and the significance indicated as $^{*}0.20 > P > 0.05$, $^{**}0.05 > P > 0.01$, $^{***}0.01 > P > 0.001$, and NS, $P > 0.20$.

Linear discriminant analysis of the respiration-normalized enzyme activities revealed two groups of responses (Fig. 5). All enzyme activities except arylsulphatase were highly correlated with each other to define the first discriminant function. These respiration-normalized activities increased from the large N treatments on the right of the biplot in Fig. 5 to the large C and detritus treatments on the left of the biplot. This indicates relative down-regulation of these enzymes possibly because of a C limitation to enzyme biosynthesis in the presence of a large N supply. Respiration-normalized arylsulphatase activity was largely uncorrelated with the other enzyme activities, except β-glucosidase with which it was only weakly negatively correlated. The normalized arylsulphatase activity defined the second discriminant function with its activity increasing from control and small C treatments at the bottom of the biplot to the large C treatments at the top of the biplot, regardless of N level. β-Glucosidase was partly correlated with both discriminant functions (its net correlation lying on a diagonal) with its maximum rate per unit respiration being for detritus treatments, indicative of enhanced enzyme activity where the detritus either supplies microbial resources in a more diverse and complex form or is directly the source of the enzyme activity.

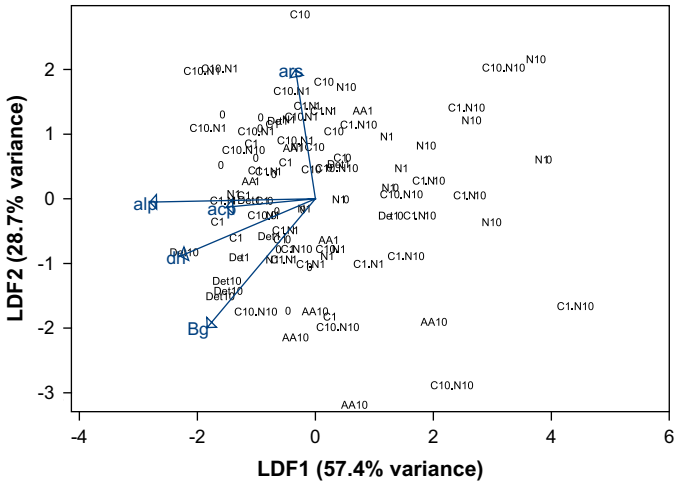

Fig. 5. Linear discriminant analysis of responses after 3 years of the five soil enzyme activities normalized for differences in respiration rate to organic C and N amendments in the Garwood Valley, Antarctica. All enzyme activity values were square-root or log-transformed prior to analysis. Individual plots are shown according to their treatment codes abbreviated as in Table 1. Biplot correlations of the five enzymes' activities are shown as vectors from the origin, with length exaggerated by a factor of 3 for clarity. Enzyme codes are: acp, acid phosphatase; alp, alkaline phosphatase; ars, arylsulphatase; Bg, β- glucosidase; dh, dehydrogenase.

3.4. Microbial community structure

Plots of the principal component scores for the ELFA data provided no evidence that the treatments had affected the microbial community structure (data not shown). ANOVAs on the first and second principal components (which accounted for 42 and 23%, respectively, of the variation) confirmed no treatment effects on the microbial community structure (data not shown). Thus the respiration and enzyme responses occurred whilst the microbial community remained apparently structurally stable, or unresponsive, at least at the level of resolution offered by ELFA analysis.

4. Discussion

Detection of respiration in soil from Garwood Valley soil is consistent with our previous results from field and laboratory measurements at the same site (Elberling et al., 2006; Hopkins et al., 2006b) as well as other dry valley soils (Burkins et al., 2002; Parsons et al., 2004; Barrett et al., 2006).

A limited amount of data already exist on biogeochemical transformations of C and N in dry valley soils (Barrett et al., 2002, 2005, 2007; Parsons et al., 2004; Hopkins et al., 2006b; Gregorich et al., 2006), but by comparison with soils outside Antarctica, knowledge of C and N biogeochemical processes in dry valley soils is still very limited. As far as we are aware, there are few data on the biogeochemical transformations of P and none for S transformations in the dry valleys soils. The fact that we have been able to detect the potential for transformations of organic C, N (in the form of glycine-induced effects), P and S compounds is a significant contribution to the available knowledge. We believe, however, that the enzyme activities are the first such measurements for dry valley soils and indicate the potential for a wider range of biogeochemical transformations than has previously been reported. There are some phosphatase and arylsulphatase data reported for soils from Antarctic Islands (Pietr et al., 1983; Bölter et al., 2002; Tscherko et al., 2003). However, the Antarctic Islands have a maritime climate which leads to wetter and warmer conditions than in the dry valleys, and the presence of conspicuous photoautotrophs contributes more organic matter to the soils, and where the soils some times contain large deposits of seabird guano. Unsurprisingly,

enzyme activities in these less extreme soils were greater than we report for the Garwood Valleys, reflecting the less extreme environmental conditions and greater overall biological activity.

Dehydrogenase assessed by reduction of tetrazolium salts is an exclusively intracellular activity commonly used as a measure of overall microbial activity because it is catalysed by a number of enzymes and reflects the total oxidative potential of the microbial community (Dick, 1997). Dehydrogenases have a central role in cellular respiration, so the detection of dehydrogenase activity is consistent with the respiration measurements. Similarly, the detection of β-glucosidase activity is consistent with microbial activity in the soil. Cellulose and cellobiose, the principal substrates for β-glucosidase, are very scarce in the dry valleys, which explains why the activities we report are smaller than those typically measured in temperate soils (Eivazi and Tabatabai, 1988; Trasar-Cepeda et al., 2000; Turner et al., 2002). β-Glucosidase activity typically correlates positively with total organic C and microbial biomass C in soils (Turner et al., 2002). In soils with small clay and organic matter contents, in which the potential for enzyme immobilization on colloid surfaces is negligible, β-glucosidase is also a good general indicator of microbial activity (Kiss et al., 1972; Turner et al., 2002). In this study, β-glucosidase and dehydrogenase activities correlate, which is consistent with them both being indicators of microbial activity and C mineralization (Fig. 5). However, the fact that dehydrogenase responses to C and N addition were not all significant when considered singly, whereas β-glucosidase were significant (Fig. 4), suggests that dehydrogenase activity is a more sensitive indicator of changing resource conditions in the soil.

Unweathered, inorganic forms of P typically dominate the P stocks in dry valley soils and the amount of potentially bioavailable P is large by comparison with the small biological requirement (Blecker et al., 2006; Bate et al., 2008). The observation of greater alkaline compared with acid phosphatase activity indicates that optimization for the high soil pH (8.4) has occurred, as has been previously reported (Speir and Ross, 1978; Juma and Tabatabai, 1978).

Respiration responded positively to both C and N additions and there was an additive effect of C and N. Furthermore, the respiratory response was sensitive to simple, combined and complex forms of C and N. Respiratory responses of a similar magnitude were recorded for detritus as for C and N from glucose and NH_4Cl even though the total N supplied by the detritus was smaller whilst the amounts of C supplied were similar. This indicates provision of resources other than C and N or a more favourable physical environment in the presence of the detritus, such as the supply of nutrients other than C and N, or their supply in more readily utilizable forms or increased water-holding capacity. The activities of all enzymes except dehydrogenase were increased by C and combined large C and N additions, but either unchanged or diminished by addition of either N only or N with only small C additions in the form of glucose and NH_4Cl. This suggests that in the presence of a large amount of N, C supply for biosynthesis is limited while priority was given to C for respiration. In turn, this indicates a large energetic demand by the soil organisms in the dry valleys, possibly related to high maintenance requirements under cold, dry and potentially desiccating conditions. Bölter et al. (2002) reached a similar conclusion based on the partition of glucose C between respiration and assimilation for soils from less extreme Antarctic environments.

There was no evidence of shifts in the community structure as a result of the C and N supplementation treatments. Thus respiration and enzyme activity responses to supplementation were likely to have been the result of up- or down-regulation of specific processes or the enzymes catalyzing them in an apparently structurally stable or unresponsive microbial community.

In conclusion, the results indicate metabolic potential to transform organic C, N, P and S compounds in the Garwood Valley soil, and that microbial activity and potential were both C- and N-limited. The responses to C and N supplementation over three years occurred without any gross changes in the microbial community structure. There may be more subtle changes in community structure not detected by ELFA analysis and this is a subject for subsequent investigation. There is also evidence that the soil organisms prioritized the use of organic C to support energetic metabolism (respiration) when other nutrient limitations were alleviated. Finally, the data also indicate that addition of the detritus provides either conditions or resources leading to larger biological responses than a similar amount of C and large amounts of N in defined compounds.

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