The response of organic matter mineralisation to nutrient and substrate additions in sub-arctic soils

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Abstract

Global warming in the Arctic may alter decomposition rates in Arctic soils and therefore nutrient availability. In addition, changes in the length of the growing season may increase plant productivity and the rate of labile C input below ground. We carried out an experiment in which inorganic nutrients (NH_4NO_3 and $NaPO_4$) and organic substrates (glucose and glycine) were added to soils sampled from across the mountain birch forest-tundra heath ecotone in northern Sweden (organic and mineral soils from the forest, and organic soil only from the heath). Carbon dioxide production was then monitored continuously over the following 19 days. Neither inorganic N nor P additions substantially affected soil respiration rates when added separately. However, combined N and P additions stimulated microbial activity, with the response being greatest in the birch forest mineral soil (57 % increase in CO_2 production compared with 26 % in the heath soil and 8 % in the birch forest organic soil). Therefore, mineralisation rates in these soils may be stimulated if the overall nutrient availability to microbes increases in response to global change, but N deposition alone is unlikely to enhance decomposition. Adding either, or both, glucose and glycine increased microbial respiration. Isotopic separation indicated that the mineralisation of native soil organic matter (SOM) was stimulated by glucose addition in the heath soil and the forest mineral soil, but not in the forest organic soil. These positive 'priming' effects were lost following N addition in forest mineral soil, and following both N and P additions in the heath soil. In order to meet enhanced

microbial nutrient demand, increased inputs of labile C from plants could stimulate the mineralisation of SOM, with the soil C stocks in the tundra-heath potentially most vulnerable.

Keywords: arctic, climate change, glucose, glycine, mountain birch, nitrogen, phosphorus, priming, soil respiration, tundra-heath

1. Introduction

Due to the large amounts of C stored in arctic and sub-arctic soils (Post et al., 1982; Ping et al., 2008), changes in organic matter decomposition rates could alter atmospheric composition and therefore climate. Arctic ecosystems are already experiencing rapid rates of climate change (ACIA, 2005). Continued warming is expected to alter the physical soil environment (both temperature and moisture) and enhance mineralisation rates which, along with N deposition from anthropogenic activities, may in turn alter nutrient availability in soils (Hobbie et al., 2002; Mack et al., 2004). In addition, increases in plant productivity (Myneni et al., 1997) and changes in the distributions of different species (Tape et al., 2006) already appear to be occurring in many areas. Therefore, arctic soils are likely to experience changes in the rates of labile C flow into the rhizosphere, which may further modify decomposition rates and long-term C storage (Fontaine et al., 2004; Bradford et al., 2008; Wookey et al., 2009).

Large amounts of relatively labile C may be present in tundra soils because physical conditions, rather than substrate chemistry, are considered to limit decomposition rates (Sjögersten et al., 2003; Nowinski et al., 2008). These low rates

of decomposition may lead to the activity of free-living microbes in arctic soils being limited by the availability of nutrients, especially N (see Hobbie et al., 2002). Therefore, if global warming increases soil nutrient availability, decomposition rates may be further stimulated.

Such stimulation of decomposition seems to have occurred in an Alaskan tussock tundra, where fertilisation resulted in substantial losses of soil C (Mack et al., 2004; Nowinski et al., 2008). During this long-term experiment (established in 1981), the plant community became increasingly dominated by deciduous shrubs, especially *Betula nana*, making it difficult to determine the mechanisms underlying the overall loss of soil C. Sullivan et al. (2007) suggested that a reduction in fine root production, associated with increased *B. nana* abundance, could explain 20-40% of the loss in C. However, as decomposition rates can be stimulated by both N and P additions (Stotzky and Norman, 1961; Fierer et al., 2003), it was concluded that the loss of C was most likely a direct response to the fertiliser treatment (Nowinski et al., 2008). It is therefore important to try and understand whether N and P additions can directly stimulate decomposition in a range of different tundra ecosystems.

Plant productivity in the Arctic is expected to increase with global warming (ACIA, 2005). Counter-intuitively, greater rates of plant productivity, or soil C input, do not necessarily result in increases in C storage below ground (Fontaine et al., 2004; Heath et al., 2005). One of the most productive arctic ecosystems, tall shrub tundra, is characterised by relatively small soil C stocks, and overall there may be a negative relationship between NPP and the proportion of ecosystem C stored below ground (ACIA 2005). Although there are many possible explanations for these patterns, including more favourable physical conditions for decomposition, both during the growing season and the winter [warmer soils under deeper snow packs (Schimel et al.,

2004; Sturm et al., 2006)], the increased mobilisation of nutrients to plant biomass may be partly the result of plant stimulation of C mineralisation rates in soils (Jonasson et al., 2006), known as positive priming (Kuzyakov, 2002). Priming theory suggests that, in soils in which nutrient availability is low, the addition of labile substrates should increase the decomposition of native SOM in order to provide the microbes with the additional nutrients they require (Cardon, 1996; Kuzyakov, 2002; Fontaine et al., 2004). Therefore, the C stocks in arctic soils may be vulnerable, especially in relatively unproductive ecosystems in which the majority of C and N stocks are located below ground (ACIA 2005).

Relative changes in labile C supply versus nutrient availability in soils will be important in determining the extent to which priming has the potential to alter carbon storage below ground. Importantly, increased plant productivity in the Arctic may occur not only in response to warming-induced increases in nutrient mineralisation, but also directly in response to climate change and increases in growing season length (Myneni et al., 1997; Euskirchen et al., 2006), with likely consequences for the distribution of different species (Euskirchen et al., 2009). If any of the increases in plant productivity are independent of, or greater than, the changes in soil-nutrient availability, then priming of SOM decomposition may occur in order to meet the enhanced plant-nutrient demand.

The greatest changes in primary productivity are likely to occur when one ecosystem invades another (Wookey et al., 2009). For this reason we carried out our experiment with soils sampled from across the mountain birch-tundra heath ecotone in northern Sweden. In this area the tundra-heath is dominated by evergreen dwarf shrubs, under which a relatively thick (8-10 cm) organic soil has developed. Although, there are no raised water tables affecting decomposition rates in these ecosystems,

approximately twice as much C is stored below-ground in the tundra heath, despite there being more plant biomass and higher plant productivity in the forest (Sjögersten and Wookey, 2009). Therefore, if decomposition rates on the heath can be enhanced by priming, any advance of the tree-line has the potential to result in a loss of soil C.

We have carried out an inorganic nutrient and organic substrate addition experiment to investigate a) whether microbial activity and C mineralisation rates are nutrient limited in these arctic soils, and b) the potential vulnerability of the C stocks to positive priming effects caused by changes in labile C inputs. We tested 1. whether C mineralisation rates in the different soils were altered by either, or both, N and P additions, 2. the effect of additions of labile-C substrates on the mineralisation rates of native soil C, 3. whether any priming effects were dependent on the nutrient status of the soils, and 4. how the responses of the soils to these treatments differed between the two ecosystems (mountain birch forest and tundra heath).

2. Materials and methods

2.1. Soil sampling

Four soil samples were removed from an area of mountain birch forest (68°19'35"N, 18°50'00"E; elevation ~520 m), and from tundra heath above the natural treeline (68°18'07"N, 18°51'16"E; elevation ~710 m), near Abisko, northern Sweden. These are the two Swedish field sites being monitored within the UK Natural Environment Research Council (NERC)-funded Arctic Biosphere Atmosphere Coupling at Multiple Scales project (ABACUS; www.abacus-ipy.org). In the mountain birch (*Betula pubescens* ssp. *czerepanovii*) forest, the understorey

vegetation is dominated by the ericaceous dwarf shrubs *Empetrum nigrum* ssp. *hermaphroditum* and *Vaccinium uliginosum*. The soil is a micro-podzol (spodosol) with a shallow, approximately 2 cm thick organic layer, an approximately 4 cm thick eluvial (albic) horizon and a light orange coloured illuvial (spodic) horizon which is not always present above coarse glacial till. We separated this soil into the organic horizon (*Birch Organic*) and eluvial mineral soil horizon (*Birch Mineral*) and investigated the effect of the substrate additions on the respiration in each. On the tundra heath, the vegetation is dominated by the evergreen dwarf shrub *Empetrum nigrum* ssp. *hermaphroditum* with some *Vaccinium vitis-idaea* and *Betula nana*. This soil has a deeper organic layer, between 5-20 cm deep (mean 11 cm), overlying coarse glacial till with large clasts and only occasional pockets of mineral soil. Therefore, only the organic horizon (*Heath*) was used for the substrate addition experiment.

2.2. Soil measurements

The soils were sieved through a 2 mm mesh with roots being removed by hand, categorised (fine roots < 1 mm, coarse roots >1 mm), dried and weighed. Sub-samples of soils were removed for moisture content, water holding capacity, and C, N and P content measurements. Moisture contents were calculated gravimetrically based on weights before and after oven drying at 105^{o}C. For WHC estimation, samples were wetted on filter paper until no more water was taken up. The samples were then dried in an oven at 105^{o}C. This new gravimetric moisture content was considered to represent WHC. The C and N contents of dried soil samples were measured by elemental analysis (Model: EA1108, Carlo Erba, Rodano, Italy). Total soil P was measured using the sodium hydroxide fusion method (Smith and Bain, 1982).

176

177 *2.3. CO_2 production and substrate additions*

178

179 The rates of CO_2 production from each soil were determined using a 96-
180 chamber respirometer (Respicond IV, Nordgren Innovations, Umeå, Sweden) which
181 allows hourly respiration measurements to be made based on conductance changes in
182 KOH traps as CO_2 is absorbed (Nordgren, 1988). Initially 11 sub-samples of each soil
183 were added to the respirometer cells (15 g subsamples for each organic soil and 45 g
184 sub-samples for each mineral soil), and the incubation temperature set to 10^{o}C. The
185 different substrates and combinations of substrates were then added to the soils. We
186 amended the soils with ^{13}C labelled D-glucose (99 atom% for all six C atoms;
187 Cambridge Isotope Laboratory Inc., MA, USA), glycine (Acros Organics, New Jersey,
188 USA), NH_4NO_3 (Fisher Scientific Ltd., Leicestershire, UK) and $NaPO_4$ (monobasic,
189 monohydrate; Acros Organics), and each pair-wise combination of the four substrates
190 (six combinations) as well as a distilled water control. The compounds were added in
191 solution with enough water to raise each soil to 50% of WHC. The organic
192 compounds were added at a rate of 15 mg of substrate C per g of soil C. To ensure
193 that equal amounts of N were added in the inorganic and organic compound additions,
194 the NH_4NO_3 was added at the rate of 8.75 mg of N per g soil C, and the $NaPO_4$ was
195 added at the rate of 8.75 mg of P per g soil C. Table 1 shows a summary of the total
196 amount of C, N and P added in each treatment. The rate of CO_2 production was
197 monitored for 19 days after substrate addition. Given that the total number of sub-
198 samples to be incubated (132) exceeded the number of chambers in the respirometer,
199 the experiment was run twice with two replicates of each soil type (e.g. *Heath* soil) in
200 each run, providing a total of four replicates per soil type. While the first set of

samples was run, the second set of samples was stored at 4^{o}C. To test whether this period of storage could have affected the overall patterns observed, we carried out a two-way ANOVA with soil type and run number as the fixed factors to test whether there was any difference between runs. In all cases (all the different substrate additions) there was no significant effect of run number ($P > 0.282$).

2.4. Isotopic measurements

To determine the proportion of CO_2 that was derived from the added glucose compared with the mineralisation of the native SOM, samples of KOH were collected at the end of the incubation for isotopic analysis. The ^{13}C content of the trapped CO_2 was determined using an ANCA-GSL sample converter coupled to a SerCon 20-20 isotope ratio mass spectrometer (SerCon Ltd, Crewe, UK). The CO_2 was released from 0.05 - 0.1 ml aliquots of KOH solution by acidification with 1 ml of 6 M orthophosphoric acid in evacuated 12 ml Exetainers™ (SerCon Ltd, Crewe, UK), prior to analysis (Hopkins et al., 2009). The contribution of glucose-derived CO_2 to the total CO_2 absorbed by the KOH during the experiment was then calculated by mass balance, based on the atom% ^{13}C of glucose-derived CO_2 (99%) and soil-derived CO_2 (1.1%). The total amount of glucose-derived CO_2 respired was then calculated based on the CO_2 accumulation results.

2.5. pH measurement

The effect of each of the single substrate additions on soil pH were measured at the end of the incubations. For the organic soils, sub-samples equivalent to 2.5 g

dry weight were weighed into plastic beakers. Distilled water was added to raise the total volume of water present up to 50 ml. For the mineral soils, 3:1 distilled water:soil slurries were produced in plastic beakers. After repeated mixing, pH values were measured using a pH meter.

2.6. Microbial biomass

Soil microbial biomass C and N was determined by chloroform fumigation-extraction based on the method developed by Brookes et al. (1985).

2.7. Statistical analysis

All statistical tests were carried out using SPSS (Version 15, SPSS Science, Birmingham, UK) and data were checked for suitability for parametric analysis (normality and equality of variance). One-way ANOVAs were used to determine whether there were significant differences between soils and/or between the different substrate addition treatments in terms of differences in soil properties, microbial biomass, total CO_2 production, respiration rates, cumulative CO_2 production and CO_2 derived from the added glucose or native SOM mineralisation. pH values were converted to H^+ concentrations before statistical analysis and the calculation of mean values. Results were considered statistically significant at the $P = 0.05$ level.

3. Results

3.1. Soil Properties

The measured soil properties are summarised in Table 2. There were obvious differences in the moisture content, C, N and P concentrations, WHC and root biomass between the organic and mineral soils. However, when the root biomasses were expressed per gram soil C, there were similar masses of fine roots in all soils, and there was more coarse-root biomass in the *Birch Organic* soil compared with the *Heath* soil. Compared with the *Heath* soil, the slightly lower P content in the *Birch Organic* soil resulted in significantly larger C:P ratio and N:P ratios. Both C:P and N:P ratios were considerably narrower in the *Birch Mineral* soil than in the two organic soils (Table 2).

3.2. Total CO_2 production

In all three soils, NH_4NO_3 addition had no effect on total CO_2 production ($P > 0.378$; Fig. 1), while, in *Birch Organic* soils only, $NaPO_4$ addition significantly reduced CO_2 production ($P = 0.001$). When the results from all three soils were considered, the addition of both inorganic nutrients together significantly increased total CO_2 production relative to the controls ($P = 0.033$), with total CO_2 production increasing by 26%, 8% and 57% in the *Heath*, *Birch Organic* and *Birch Mineral* soils, respectively. However, within each soil, these trends were not quite statistically significant ($P > 0.083$).

Total CO_2 production increased significantly when glucose ($P < 0.011$) and glycine ($P < 0.002$) were added (Fig. 1), with glycine addition resulting in significantly more CO_2 production than glucose addition ($P < 0.019$). The addition of glucose and glycine together increased CO_2 production beyond that observed for

276 either substrate separately (*Heath*: $P < 0.003$, *Birch Organic*: $P < 0.010$), although the
277 increase was not statistically significant in the *Birch Mineral* soil ($P = 0.097$).
278 The addition of the inorganic nutrients together with organic substrates had
279 little effect on CO_2 production compared with adding organic substrates on their own.
280 However, there were two exceptions; for the *Birch Organic* soil, glucose and NH_4NO_3
281 addition increased CO_2 production beyond the addition of glucose alone ($P = 0.004$),
282 and in the *Heath* soil, adding NH_4NO_3 together with the glycine reduced CO_2
283 production in comparison with just adding glycine ($P = 0.002$).
284 There were relatively few significant differences in total CO_2 production
285 between the three soils. When $NaPO_4$ was added CO_2 production was greater in the
286 *Birch Mineral* soil than the *Birch Organic* soil ($P = 0.018$), while there was a clear
287 difference between soils when glycine and NH_4NO_3 were added together. Under this
288 treatment, total CO_2 production from the *Heath* soil was significantly lower than from
289 the *Birch Organic* ($P < 0.001$) and *Birch Mineral* soil ($P < 0.001$).
290
291 *3.3. Respiration rates*
292
293 For the control, NH_4NO_3 and $NaPO_4$ additions, rates of CO_2 production were
294 relatively stable throughout the incubation period (Fig. 2). For the organic substrates,
295 the timing of peak respiration rates differed between glucose and glycine additions
296 and between the different soils. Glucose addition had an immediate effect on CO_2
297 production with respiration rates being greatest within the first 24 hours of incubation.
298 In contrast, under glycine addition, peak respiration rates were observed after 8-11
299 days in the *Birch Organic* and *Birch Mineral* soils, while respiration rates increased
300 throughout the incubation in the *Heath* soil (Fig. 2). When glycine and NH_4NO_3 were

added together respiration rates were generally greater in the two birch soils until day 15, but the timing of peak CO_2 production appeared to be delayed slightly (until days 11 to 13) in the *Birch Mineral* soil. The differences in respiration rates between soils were lost when glycine and $NaPO_4$ were added together.

3.4. Isotopic partitioning of respired CO_2

The addition of 99 atom % ^{13}C labelled glucose made it possible to partition the collected CO_2 into that derived from the mineralisation of SOM, and that respired from the added glucose. When all three soils were considered together, the increase in the amount of CO_2 respired over the incubation period following glucose addition came from both the added glucose and a significant increase in SOM mineralisation ($P = 0.038$). Overall, glucose addition stimulated the mineralisation of SOM by 27%, but there appeared to be differences between soils. The stimulation was greatest in the *Birch Mineral* soil (54 %), smaller in the *Heath* soil (31 %) and not significant (-3 %) in the *Birch Organic* soil (Fig. 3). The total amount of CO_2 produced was not affected by combining the glucose with the NH_4NO_3 or $NaPO_4$ additions ($P = 0.884$). However, NH_4NO_3 addition altered the source of the respired CO_2; a greater proportion of the CO_2 came from the glucose ($P < 0.001$), eliminating the priming effect. This was not the case for the $NaPO_4$ additions ($P = 0.256$). The absolute amount of CO_2 derived from glucose was significantly increased when glycine was also added ($P < 0.001$).

Within the individual soil types, although there were fewer statistically-significant differences compared with the overall patterns (perhaps due to the lower statistical power), there appeared to be differences in the trends observed between the different soils. In all three soils, NH_4NO_3 addition increased the amount of CO_2

derived from glucose (Fig. 3a). In contrast, $NaPO_4$ addition only enhanced glucose-derived CO_2 production in the *Heath* samples ($P = 0.027$). For the *Heath* soil, given that the total amounts of CO_2 produced following glucose addition were nearly identical, irrespective of whether or not nutrients were added (Fig. 1a), the significant increase in glucose-derived CO_2 strongly suggests that SOM mineralisation was reduced by adding both NH_4NO_3 and $NaPO_4$ in conjunction with glucose, in comparison with adding glucose alone. For the *Birch Organic* samples, NH_4NO_3 addition significantly increased CO_2 production from the glucose, but did not reduce SOM mineralisation, and thus significantly increased total CO_2 production (Fig. 1b; $P = 0.004$). The fact that NH_4NO_3 addition in conjunction with glucose did not reduce SOM mineralisation is perhaps not surprising given that when glucose was added on its own no increase in SOM mineralisation was observed (Fig. 3b). In the *Birch Mineral* soil, a non-significant trend towards the positive priming of SOM mineralisation by glucose addition, which was subsequently lost on NH_4NO_3 addition, was observed, while $NaPO_4$ addition appeared to have no effect on SOM mineralisation (Fig. 3). Despite the different patterns, overall, the only significant difference observed between the different soils was a significantly greater respiration of glucose-derived CO_2 in the *Birch Organic* soil when NH_4NO_3 was also added ($P = 0.020$).

For the *Heath* soil, adding the glucose with either N or P increased cumulative CO_2 production between days 5 and 8 compared with adding glucose on its own (Fig. 4). Subsequently, a greater rate of respiration in the soils amended with glucose alone resulted in the differences between treatments being lost. Furthermore, cumulative CO_2 production between days 5 and 19 was 1.2 mg C g soil C^{-1} greater in the glucose addition treatment than in the control (Fig. 4). The glucose treatment was

the only treatment in which positive priming effects were detected, while more glucose-derived CO_2 was produced when the glucose was added together with the nutrients. Therefore, the differences in the temporal patterns of cumulative CO_2 production suggest that the increase in glucose-derived CO_2 production caused by the inorganic nutrient additions occurred early on in the incubation, while the majority of positive priming effects probably occurred late on in the incubation.

3.5. pH results summary

We tested the effect of substrate addition on soil pH. Glycine addition substantially increased soil pH in all three soils (Table 3; $P < 0.031$). In contrast, glucose addition had little effect on soil pH, never altering it by more than 0.1 pH units ($P > 0.061$). The addition of NH_4NO_3 significantly reduced pH values in all three soils ($P < 0.040$) and $NaPO_4$ addition also tended to reduce soil pH, but only significantly in the case of the *Birch Organic* soil ($P = 0.013$). The *Heath* soil had a significantly higher pH than both the *Birch Organic* ($P = 0.003$) and *Birch Mineral* soils ($P = 0.041$).

3.6. Microbial biomass

When expressed per gram of soil, microbial biomass C and N did not differ significantly between the three soils (Table 4). However, the C:N ratio of the microbial biomass in the *Heath* soil was significantly greater than in both birch soils (Table 4; $P < 0.001$).

4. Discussion

4.1. The effect of inorganic nutrient additions on C mineralisation rates

When added singly, neither NH_4NO_3 nor $NaPO_4$ increased total CO_2 production. In fact, $NaPO_4$ addition significantly decreased CO_2 production from the *Birch Organic* soils. It would appear that respiration rates were relatively insensitive to changes in the concentrations of single nutrients and therefore may respond little to anthropogenic N deposition, at least in the short term.

There have been a number of studies which, like ours, monitored the effect of nutrient additions on soil CO_2 production in the laboratory. In broad agreement with the results of our study, working with arctic soils, Yoshitake et al. (2007a) found that N additions only stimulated respiration rates in early successional, but not in late successional, glacial soils. In the literature, N additions have been shown to have inhibitory (Söderström, 1983; Fog, 1988; Cheshire and Chapman, 1996; Fierer et al., 2003; Bradford et al., 2008; Ouyang et al., 2008), neutral (Sjöberg and Persson, 1998; Yoshitake et al., 2007b), and stimulatory (Cheshire and Chapman, 1996; Fierer et al., 2003) effects, with the response differing with soil depth in the Fierer et al. (2003) study; respiration was inhibited in the surface soil horizons but stimulated substantially at depths below 25 cm. Demonstrating the importance of existing soil nutrient availabilities, Amador and Jones (1993) and Cheshire and Chapman (1996) found that N additions inhibited plant residue decomposition or respiration rates in peat soils, respectively, only when the natural P availability was low, but had neutral or stimulatory effects when P availability was high.

Weintraub and Schimel (2003) found that, whilst there was no decline in respiration rates during a long-term incubation of wet meadow, tussock and intertussock soils, C mineralisation rates declined substantially in shrub tundra soils. From this they concluded that the pool of actively decomposing C (labile pool) only represented approximately 15% of total SOM in these soils. Our shrub-tundra soils were dominated by evergreen dwarf shrubs rather than the high-stature deciduous shrubs present in the Weintraub and Schimel (2003) study, and it has been found that that evergreen litters tend to decomposed more slowly (Cornelissen, 1996; Cornelissen et al., 2007). The accumulation of relatively recalcitrant, lignin-rich SOM may explain the presence of the thick organic horizon at our tundra-heath site, despite the absence of a raised water table. Weintraub and Schimel (2003) also demonstrated that the shrub tundra soils had the greatest acid-insoluble fraction (lignin-containing fraction) and that the ligno-cellulose ratio tended to decline with incubation time, suggesting that there was active lignin decomposition in these soils. As higher N availability may result in the formation of more recalcitrant compounds during lignin degradation, and reduce the overall rate of lignin breakdown (Berg, 2000), this may explain why we did not observe an increase in respiration rates following N addition to our soils.

In summary, our results, and those of other studies, suggest that N additions do not consistently stimulate soil respiration rates, even in ecosystems characterised by low N availability, and that the type of SOM present may be important in determining the response of microbial respiration rates.

For P additions, in contrast to our results, a number of studies have reported stimulatory effects on respiration rates (Amador and Jones et al., 1993; Cheshire and Chapman, 1996; Fierer et al., 2003; Bradford et al., 2008; Ouyang et al., 2008), with

few negative results observed. Amador and Jones et al. (1993) found that P additions only stimulated activity when the natural P availability was low. However, it should be noted that in our experiment P was added in the form of $NaPO_4$ whilst in these other studies it was added in the form of K_2HPO_4. This could also explain some of the differences observed between our results and previous work. A systematic investigation into the response of microbial communities and decomposition rates to changes in nutrient availability in soils differing in their geographical area, climate, native plant community and underlying bedrock may be required to generate a fuller understanding of responses.

In our study, although neither N nor P additions increased C mineralisation rates when added separately, adding the two inorganic nutrients together increased respiration rates in all three soils, with the response being greatest in the *Birch Mineral* soil. Fierer et al. (2003) also found that C mineralisation rates in sub-surface soils were enhanced more when N and P were added together, and our results are consistent with those of Amador and Jones (1993) who demonstrated that N additions only increased decomposition rates when P availability was high. Bradford et al. (2008) demonstrated that, in terms of the stimulation of decomposition by P addition, it was the mineral-associated organic matter which was most responsive. This may also explain why, in our study, it was respiration in the *Birch Mineral* layer which increased most in response to the combined N and P additions. In addition, this work also suggests that the role of P in stimulating decomposition in fertilisation experiments, in which it has been added in conjunction with N (e.g. Mack et al., 2004), could have been underestimated. Overall, it appears that N and P availability combined to limit microbial respiration in our experiment.

4.2. Priming of SOM mineralisation and the respiration of glucose-derived C

Priming theory suggests that positive priming effects should be most pronounced in soils characterised by low nutrient availability (Cardon, 1996; Kuzyakov, 2002; Fontaine et al., 2004), especially N (Craine et al., 2007). For these reasons, we hypothesised that the mineralisation of SOM in sub-arctic soils may be especially responsive to priming by glucose additions. In support of this suggestion, overall, SOM-derived CO_2 production was enhanced by approximately 27% following glucose addition and this priming effect was lost following N addition (Fig. 3).

There appeared to be differences between the three soils in terms of the effects of glucose addition on SOM mineralisation. No priming of SOM decomposition was observed for the *Birch Organic* soil (Fig. 3). In the *Heath* soil and *Birch Mineral* soil, SOM mineralisation rates were greater under glucose addition, with priming effects being lost following N addition in both soils. In the *Heath* soil, it also appeared that priming effects were reduced by P addition. Therefore, and in contrast to previous work (Craine et al., 2007), some of the priming response may have been associated with increased SOM mineralisation to meet microbial P demand in the *Heath* soil. This occurred despite the fact that there was a higher total P content and narrower C:P and N:P ratios in the *Heath* soil compared with the *Birch Organic* soil (Table 2).

It has been suggested that, rather than representing an increase in the rate of SOM turnover, apparent positive priming effects may be caused by (1) the stimulation microbial metabolism by trace amounts of labile C (De Nobili et al., 2001; Blagodatskaya and Kuzyakov, 2008), or (2) an acceleration of microbial biomass turnover in the medium term (Blagodatskaya et al., 2007; Blagodatskaya and

475 Kuzyakov, 2008). In our experiment, as the amount of glucose-C added was
476 approximately twice the size of the microbial biomass-C pool, and the priming effects
477 appeared to occur late on in the incubation (Fig. 4), it is highly unlikely that the first
478 explanation caused the positive priming effect we observed.

479 Although we were not able to make continuous measurements of microbial
480 biomass during our experiment, the level of glucose that we applied will probably
481 have resulted in an initial increase in microbial biomass followed by a subsequent
482 decline (Schneckenberger et al., 2008), and therefore increased microbial biomass
483 turnover, potentially contributing to the positive priming effect observed. However, in
484 our experiment, priming effects were eliminated by concurrent additions of mineral
485 nutrients (see above). This suggests that when glucose was added alone, the increase
486 in SOM-derived CO_2 production was the result of increased microbial nutrient
487 demand, and that the priming effects were real, rather than apparent.

488 In summary, it appears that rates of native SOM decomposition can be
489 enhanced by labile C inputs in the *Heath* and *Birch Mineral* soils. Therefore, changes
490 in plant productivity, or the distribution of different plant communities, could
491 stimulate rates of decomposition, making the *Heath* soil C stores potentially
492 vulnerable. Weintraub and Schimel (2003) demonstrated that there was a large
493 amount of recalcitrant SOM present in shrub tundra soils, but that microbial activity
494 in wetter tundra soils was limited by a factor other than substrate availability, with
495 substrate availability being very high relative to microbial biomass. Therefore, in
496 wetter ecosystems, carbon stocks may be less vulnerable to priming as a result of
497 increased labile C inputs, and our results are relevant mainly to mesic shrub tundra,
498 especially that dominated by evergreen shrubs. In addition, as mentioned in the
499 introduction, warming-induced increases in rates of nutrient mineralisation in soils

may partly meet the increased plant nutrient demand, potentially reducing the importance of priming effects in a warmer Arctic.

The net effect of labile C addition on SOM stocks will be dependent on the extent to which native SOM decomposition is stimulated versus the extent to which the added C is converted into SOM. Bradford et al. (2008) demonstrated that even labile substrates such as sucrose may be modified by microbial metabolism and contribute to new SOM formation. They found that the amount of substrate added controlled whether or not the net effect on total C storage was positive or negative, and that the formation of new SOM from the sucrose was enhanced by N and P additions. Although directly determining the impacts of the different treatments on C storage and new SOM formation would have required a much longer-term experiment, we found that amount of C respired from the glucose was significantly enhanced by N addition; enhanced glucose-derived CO_2 production could potentially reduce the amount of new SOM formed from the added substrate. Alternatively, there may have been an increase in the total amount of glucose utilised (both for respiration and biomass production) which may also result in more glucose being converted into new SOM in the medium term.

Finally, in terms of climate change impacts, it should be mentioned that even if priming results in increases in above-ground plant biomass which balance any soil C losses, greater plant biomass will reduce summer albedo, and result in greater heat absorption and a positive feedback to climate change (Euskirchen et al., 2009).

4.3. Response to glycine addition

524 Glycine additions stimulated respiration rates for a longer time period than
525 glucose additions did, and this resulted in more CO_2 being produced over the course
526 of the whole incubation. This is in agreement with previous studies in which amino
527 acid additions have tended to stimulate microbial respiration rates to a greater extent
528 than glucose additions (O'Dowd and Hopkins 1998; Meli et al., 2003). The simplest
529 explanation for this result is the fact that glycine is a source of both C and N.
530 However, when the same amounts of C and N were added in the combined glucose
531 and NH_4NO_3 additions, respiration rates were still not stimulated as much as when
532 glycine was added. In tundra soils, mineral N availability may be low, and amino
533 acids may be a more abundant source of N (Weintraub and Schimel, 2005; Näsholm
534 et al. 2009). Nitrogen mineralisation is typically extremely slow in the acidic soils
535 present around Abisko (Bjork et al., 2007). Furthermore, amino acid pools have been
536 shown to be highly dynamic, even at low temperatures (Weintraub and Schimel,
537 2005; Kielland et al., 2007). Therefore, the strong stimulation of respiration rates by
538 the glycine addition may be partly due to the fact that the C and N were being added
539 in a form that the microbes are adapted to utilising.
540 Although there was no isotopic tracer applied with the glycine, the magnitude
541 of the increase in CO_2 production implies that SOM mineralisation was enhanced. The
542 rate of addition was 15 mg C g soil C^{-1}, and CO_2 production was stimulated by
543 between 12.3 and 14.4 mg C g soil C^{-1} in the different soils (Fig. 1). It is extremely
544 unlikely that all the glycine was mineralised to CO_2 during the course of the
545 experiment and therefore SOM mineralisation was probably enhanced. However,
546 priming theory suggests that adding a substrate with a narrow C:N ratio may reduce
547 the rate of SOM decomposition; the preferred substrate hypothesis proposes that if
548 microbes shift to utilising the new substrate, or the microbes utilising the new

549 substrate start to out-compete those involved in C mineralisation, then the rate of
550 native SOM turnover will decrease (Kuzyakov, 2002). Interpreting the results of the
551 glycine addition in terms of priming effects is complicated by the change in soil pH.
552 As soil pH increased by nearly one whole pH unit, the change in the physico-chemical
553 environment may well be involved in the probable stimulation of SOM mineralisation
554 (Table 3). The changes in pH were likely caused by ammonification (Xu et al. 2006),
555 and therefore soil pH may have risen gradually during the course of the experiment, at
556 least partially explaining why the peak in CO_2 production associated with glycine
557 addition occurred relatively late in the incubation period (Fig. 2). Furthermore,
558 differences between soils in terms of temporal changes in pH may have been involved
559 in the different patterns of CO_2 production from the *Heath* versus the *Birch Organic*
560 and *Birch Mineral* soils.

561 The patterns observed in response to glycine addition provide further evidence
562 for differences between the soils in terms of the N versus P limitation. Firstly, when
563 glycine and N were added together in the *Heath* soil, total CO_2 production was
564 reduced compared with adding glycine on its own; no such response was observed in
565 the two birch soils. We suggest that this may have been because microbial respiration
566 was limited by both N and P availability in the *Heath* (less glycine was mineralised
567 when inorganic N was also added as the microbes became P-limited, rather than N-
568 limited), but that N-limitation dominated in the forest. Rinnan et al. (2007), who
569 measured thymidine incorporation to investigate which factors limit bacterial activity
570 in another area of sub-arctic heath near Abisko, also found that bacteria were limited
571 by multiple factors; when C, N and P were added together, the increase in bacterial
572 activity was much greater than for any of the single or pair-wise combinations of
573 substrates.

The between-soil differences in the responses to glycine and phosphate addition also support this suggestion. Respiration rates under glycine addition peaked between days 8 and 11 in the birch soils, but they continued to rise throughout in the *Heath* samples (Fig. 2). However, when glycine and P were added together the temporal changes in respiration rates were nearly identical between soils (Figs 2 and 3), suggesting that the delay in glycine mineralisation in the *Heath* soils could be reversed by increased P availability. This result is similar to that observed by Demetz and Insam (1999) for glucose and P additions; as P limitation was mitigated the timing of the peak in CO_2 production became earlier and the peak itself became larger. Overall, the results appear to suggest that P limitation is more important in regulating the mineralisation of labile substrates (both glucose and glycine) in the *Heath* soil than in the forest soils. These results could be explained by differences between the soils in terms of how tightly linked P availability is to organic matter turnover versus mineral weathering. The *Birch Organic* soil is only on average 2 cm thick and in continuous contact with fine-grain mineral soil. Reflecting the relatively young nature of this mineral soil, its N:P ratio is relatively narrow (Table 2). The close proximity to this potential source of P may explain why microbial activity appears not to be P limited in the *Birch Organic* soil despite the C:P and N:P ratios being wider in this soil than in the *Heath* soil. In contrast, the organic soil layer in the heath is thicker and often underlain only by coarse glacial till or large clasts (often >15 cm in long axis), and as such may be more isolated from mineral weathering.

4.4. Potential consequences for C mineralisation rates in sub-arctic soils

Our results suggest that C mineralisation rates in these soils are relatively insensitive to changes in the availability of single nutrients. However, C mineralisation rates, especially in the *Birch Mineral* soil, may increase if there is a general rise in nutrient availability. If global warming increases mineralisation rates then our results suggest that there is the potential for microbial activity to be further stimulated by enhanced nutrient availability. However, *in situ*, the additional nutrients may be taken up by plant roots and/or mycorrhizas, or immobilised in microbial biomass, thus limiting the effects on C mineralisation rates. In addition, changes in activity may be transient, and we cannot predict whether increased C mineralisation rates will occur over sufficient time periods to alter C storage.

Our results do suggest that increased rhizodeposition of labile substrates could stimulate C mineralisation especially in the *Heath* and *Birch Mineral* soils. The lack of a priming effect in the *Birch Organic* soils may reflect greater nutrient availability in these soils due to the input of higher quality leaf litter from the mountain birch trees (Sjögersten and Wookey, 2005; Sjögersten and Wookey, 2009). This appears to be reflected in the lower C:N ratio in microbial biomass extracted from the birch soils compared with the *Heath* soils (Table 4), although it does not explain the differences between the organic and mineral soil layers in the forest (both in terms of priming effects and the response to inorganic nutrient additions). Overall, we conclude that the C stocks in the heath may be more vulnerable to changes in plant productivity and rates of labile C input.

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794

Table Legends

Table 1. The total amount of C, N and P added to the soils in the eleven different substrate-addition treatments.

Table 2. Summary of the analyses made on the different soils. Root biomasses are expressed in terms mg dry weight per gram soil dry weight or per gram soil C. Mean values ±1SE are displayed in each case. Within an analysis (row), soils labelled with different letters differ significantly.

Table 3. The mean pH values for each soil and each substrate addition. Within a soil (row), substrates labelled with different letters differ significantly. Statistical analyses were carried out on, and mean values calculated from, H^+ concentrations rather than pH values.

Table 4. Microbial biomass C and N in the different soils expressed per gram dry weight and per gram of soil C. Mean values ±1SE (n = 4) are shown. Within a microbial biomass category (row), soils labelled with different letters differ significantly.

815 **Figure Legends**

816

817 Fig. 1. Total CO_2 production after 19 days of incubation at 10^{o}C, for each substrate
818 and combination of substrates, in (a) *Heath*, (b) *Birch Organic* and (c) *Birch Mineral*
819 soils. Error bars represent ±1SE (n = 4). Within a soil (panel), bars labelled with
820 different letters differ significantly.

821

822 Fig. 2. Changes in the rate of respiration over the 19 day incubation period, in each of
823 the three soils for each substrate-addition treatment. Mean values ±1SE are shown
824 (n = 4). Significant differences between soils are indicated with "*".

825

826 Fig. 3. The amount of CO_2 produced (a) from the added glucose, and (b) from SOM
827 decomposition, under the glucose additions, in conjunction with the mineral nutrient
828 additions and in comparison with the control. Error bars represent ±1SE (n = 4).
829 Within a panel and soil, bars labelled with different letters differ significantly.

830

831 Fig. 4. Cumulative CO_2 production over the 19 day incubation period in the *Heath*
832 soil samples which experienced the different glucose addition treatments. Mean
833 values ±1SE are shown (n = 4). Significant differences between the three glucose
834 addition treatments are indicated with "*".

835

836

Table 1.

	Total addition (mg g soil C^{-1})			
Substrate Addition	C	^{13}C	N	P
Control (dH_2O)	0	0	0	0
NH_4NO_3	0	0	8.75	0
$NaPO_4$	0	0	0	8.75
NH_4NO_3 + $NaPO_4$	0	0	8.75	8.75
Glucose	15	14.8	0	0
Glucose + NH_4NO_3	15	14.8	8.75	0
Glucose + $NaPO_4$	15	14.8	0	8.75
Glycine	15	0	8.75	0
Glycine + NH_4NO_3	15	0	17.5	0
Glycine + $NaPO_4$	15	0	8.75	8.75
Glycine + Glucose	30	14.8	8.75	0

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Table 2.

	Heath	*Birch Organic*	*Birch Mineral*
C content (% dry wt.)	44.6±1.56 [a]	48.6±2.34 [a]	2.97±0.59 [b]
N content (% dry wt.)	1.41±0.089 [a]	1.61±0.107 [a]	0.109±0.021 [b]
P content (% dry wt.)	0.159±0.008 [a]	0.139±0.011 [a]	0.031±0.002 [b]
C:N ratio	31.9±2.52 [a]	30.4±0.60 [a]	27.0±1.03 [a]
C:P ratio	283±22.9 [b]	353±16.9 [a]	99.2±22.5 [c]
N:P ratio	8.87±0.263 [b]	11.6±0.506 [a]	3.65±0.818 [c]
Moisture (%)	74.5±0.42 [a]	69.0±1.15 [b]	20.8±0.65 [c]
Water holding capacity (%)	84.9±1.61 [a]	82.6±1.72 [a]	36.5±6.65 [b]
Fine root biomass (mg DW g DW^{-1})	32.0±4.73 [a]	27.5±8.30 [a]	1.67±0.58 [b]
Fine root biomass (mg DW g C^{-1})	64.6±8.70 [a]	53.0±15.49 [a]	56.3±14.85 [a]
Coarse root biomass (mg DW g DW^{-1})	62.5±11.6 [b]	102±13.1 [a]	1.38±0.55 [c]
Coarse root biomass (mg DW g C^{-1})	125±21.1 [b]	197±31.0 [a]	46.0±14.1 [c]

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Table 3.

	pH Values				
	Control	NH_4NO_3	$NaPO_4$	Glucose	Glycine
Heath	4.8 ^{b}	4.0 ^{c}	4.3 ^{b}	4.8 ^{b}	5.9 ^{a}
Birch Organic	4.0 ^{b}	3.4 ^{d}	3.6 ^{c}	4.0 ^{b}	5.6 ^{a}
Birch Mineral	4.2 ^{b}	3.3 ^{c}	4.2 ^{ab}	4.1 ^{b}	4.7 ^{a}

846

847

848

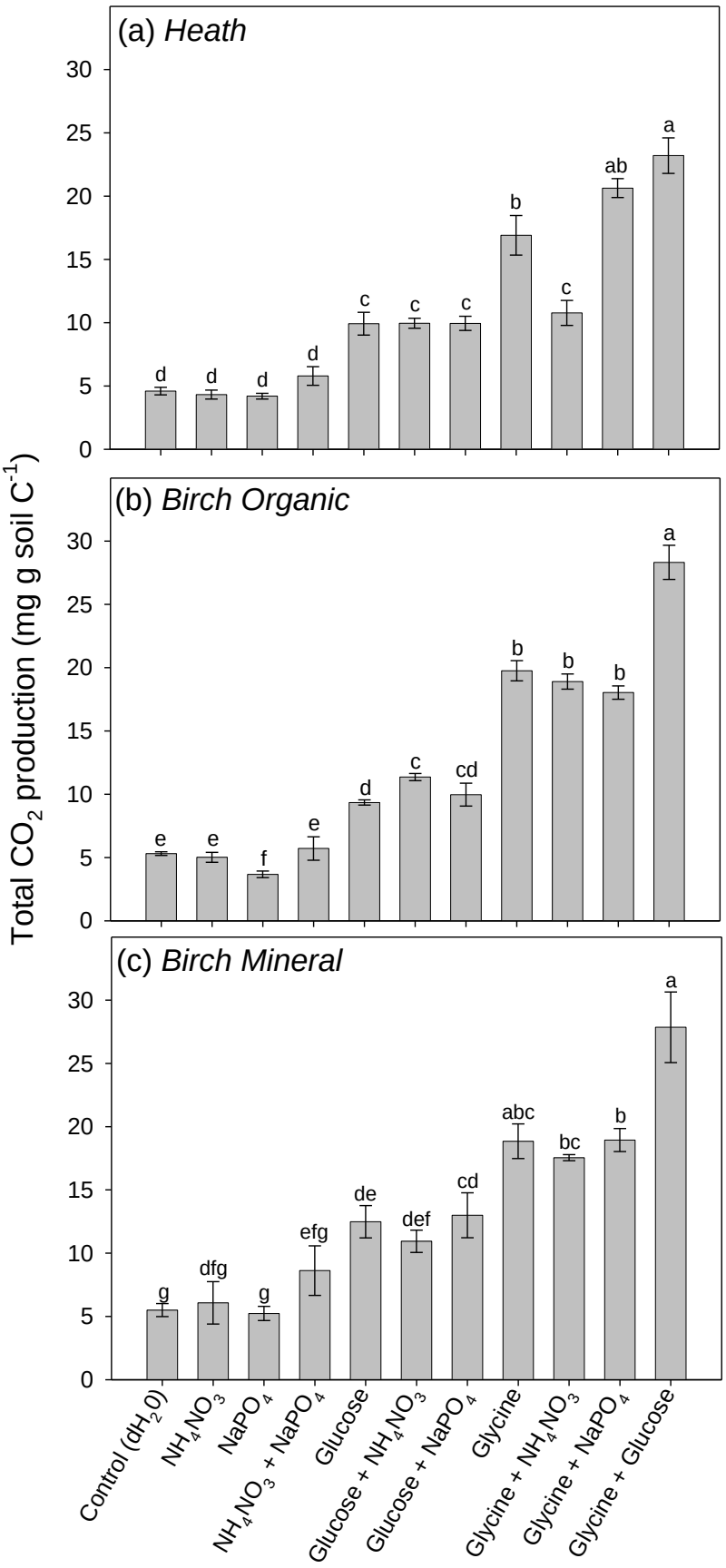
Table 4.

Assay	*Heath*	*Birch Organic*	*Birch Mineral*
Microbial Biomass C (mg g C^{-1})	7.8±0.42	6.1±0.53	7.3±0.76
Microbial Biomass N (mg g C^{-1})	1.6±0.14	1.7±0.11	2.0±0.19
C:N ratio	4.9±0.20 [a]	3.5±0.10 [b]	3.8±0.18 [b]

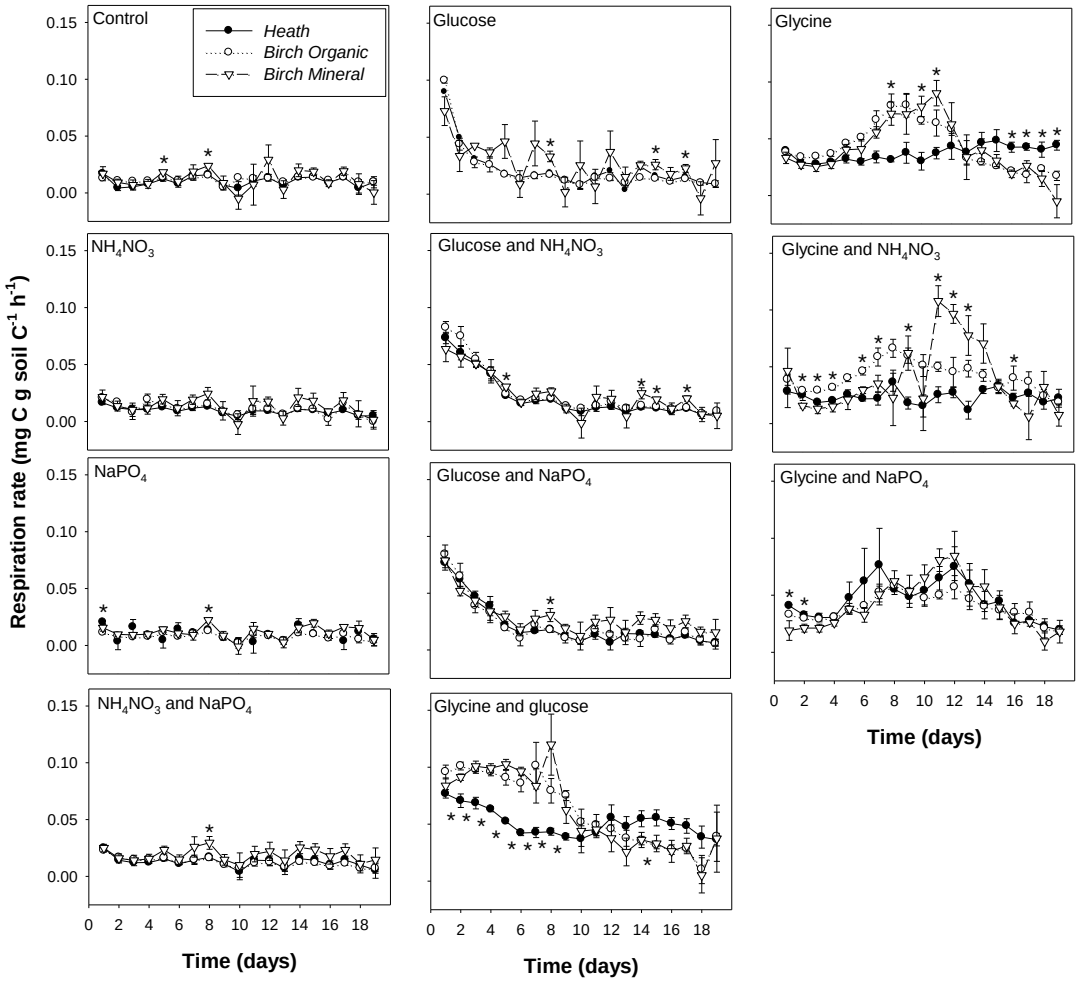
849

850

851 Fig. 1.


(a) Heath
(b) Birch Organic
(c) Birch Mineral
Total CO2 production (mg g soil C-1)
0
5
10
15
20
25
30
d d d d c c c b c ab a
e e f e d c cd b b b a
g dfg g efg de def cd abc bc b a
Control (dH20)
NH4NO3
NaPO4
NH4NO3 + NaPO4
Glucose
Glucose + NH4NO3
Glucose + NaPO4
Glycine
Glycine + NH4NO3
Glycine + NaPO4
Glycine + Glucose

852 Fig. 2.



853

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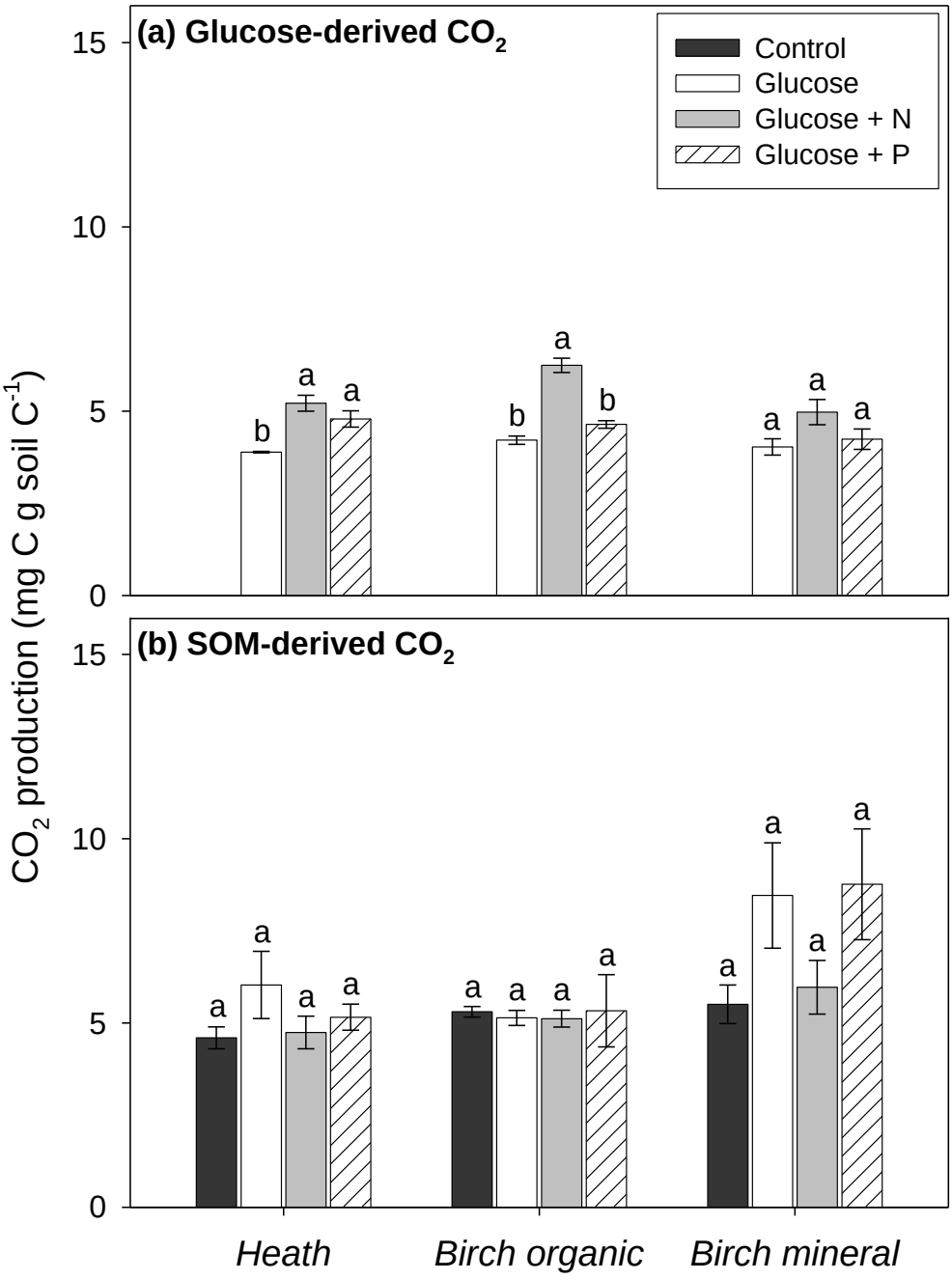
855 Fig. 3.



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862 Fig. 4.

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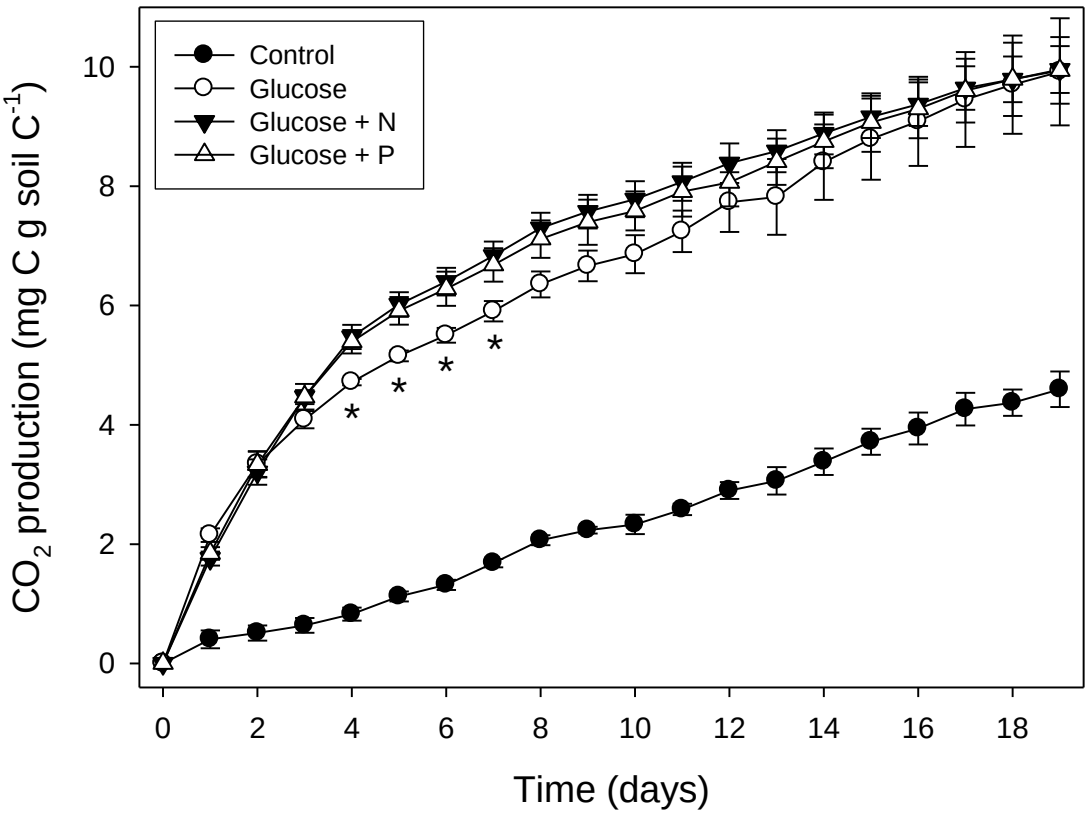

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