

# Warming prevents the elevated CO<sub>2</sub>-induced reduction in available soil nitrogen in a temperate, perennial grassland

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## Abstract

Rising atmospheric carbon dioxide concentration ([CO<sub>2</sub>]) has the potential to stimulate ecosystem productivity and sink strength, reducing the effects of carbon (C) emissions on climate. In terrestrial ecosystems, increasing [CO<sub>2</sub>] can reduce soil nitrogen (N) availability to plants, preventing the stimulation of ecosystem C assimilation; a process known as progressive N limitation. Using ion exchange membranes to assess the availability of dissolved organic N, ammonium and nitrate, we found that CO<sub>2</sub> enrichment in an Australian, temperate, perennial grassland did not increase plant productivity, but did reduce soil N availability, mostly by reducing nitrate availability. Importantly, the addition of 2 °C warming prevented this effect while warming without CO<sub>2</sub> enrichment did not significantly affect N availability. These findings indicate that warming could play an important role in the impact of [CO<sub>2</sub>] on ecosystem N cycling, potentially overturning CO<sub>2</sub>-induced effects in some ecosystems.

**Keywords:** biogeochemistry, elevated CO<sub>2</sub>, FACE, progressive nitrogen limitation, warming

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## Introduction

Increasing atmospheric concentrations of carbon dioxide (CO<sub>2</sub>) due to fossil fuel emissions have the potential to stimulate primary productivity in terrestrial ecosystems, thereby increasing the ability of these ecosystems to sequester carbon (C) and act as C sinks. Long-term field experiments have shown that this CO<sub>2</sub> fertilization effect may be limited by the availability of soil nitrogen (N) (Luo *et al.*, 2004; Reich *et al.*, 2006a), defined here as the sum of ammonium, nitrate and the low molecular weight (MW) fraction of dissolved organic nitrogen (DON) (Jones *et al.*, 2004, 2005). At elevated CO<sub>2</sub>, an increase in C fixation can result in an enhanced sink for N in the soil biota; this immobilization of N may lead to a reduced CO<sub>2</sub>-fertilization effect in a process termed progressive N limitation (PNL) (Luo *et al.*, 2004). Global temperatures are also increasing along with the increase in atmospheric CO<sub>2</sub> concentrations (Houghton *et al.*, 2001). While evidence from free air CO<sub>2</sub>-enrichment (FACE) experiments exists to justify PNL (Reich *et al.*,

2006c), there is uncertainty as to the effects that global warming will have on soil N availability and whether increasing soil temperatures will exacerbate or reverse the elevated CO<sub>2</sub>-induced reduction in soil available N (Loiseau & Soussana, 2000).

Biogeochemical cycles are complex, involving many different chemical forms and organisms (Wieder *et al.*, 2004). Because temperature is a key regulator of both chemical and enzymatic processes, global warming is extremely likely to alter the rates of conversion between chemical species, especially if the various conversion stages have differing sensitivity to temperature (Fenner *et al.*, 2006). Further, some of the chemical conversions in the soil are substrate limited, at least for N transformations (Robertson & Groffman, 2007). Experimental warming is known to increase the rate of N mineralization (Peterjohn *et al.*, 1994), potentially removing the substrate limitation on some soil nutrient transformations. Whether warming interacts with an increase in [CO<sub>2</sub>] to change various nutrient transformation rates, thereby affecting nutrient availability to plants, is so far unknown.

Here we present data from a native, perennial grassland ecosystem in Tasmania, Australia that has been

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exposed to both FACE and warming treatments for 5 years. Specifically, we investigate the potential interactions between warming and elevated CO<sub>2</sub> on soil N availability.

## Materials and methods

### Study site and experimental design

Data were collected from the TasFACE climate change impacts experiment at Pontville in southeastern Tasmania, Australia (42°42'S 147°16'E, 40 m a.s.l.). This experiment exposes a species-rich temperate grassland to both pure-CO<sub>2</sub> FACE and infrared warming. Experimental treatments commenced in February 2002 and currently continue. Full details of the experimental design and system performance were provided in Hovenden *et al.* (2006). Briefly, the experiment consists of 12 mini-FACE rings of 1.4 m diameter, in which vegetation is exposed to either ambient or an elevated [CO<sub>2</sub>] of 550 µmol mol<sup>-1</sup> (termed control and FACE plots), and are either warmed or unwarmed. Thus, the experiment is a factorial 2 × 2 design with three replicate plots of each CO<sub>2</sub> × warming combination. Warming is provided by the addition of 140 W m<sup>-2</sup> of infrared radiation using 240 V 250 W Emerson solid ceramic infrared emitters suspended 1.2 m above the soil surface above the centre of each ring and operating continuously, providing an average warming of leaf temperature of 1.98 °C and of soil temperature at 1 cm depth by 0.82 °C over the growing season. The warming treatment also reduces mean relative humidity within the canopy (at 5 cm) by an average of 5.1% over the same period. Infrared warming is dependent upon distance from the emitting surface and thus tall plants are warmed more than short plants, however in this short grass pasture, the variation in plant height is only 5–10 cm, so these differences are minor. Further, while this design does not provide completely uniform elevation of canopy temperature, 92% of the plot area is elevated by between 1.5 and 2.5 °C. Soil water potential is estimated using two gypsum blocks (ICT International, Armidale, Australia) per plot inserted at 5–15 cm depth, with water potential readings recorded weekly.

The vegetation at the site is species rich, containing approximately 64 vascular plant species. The vegetation is dominated by two perennial grass species, the C<sub>4</sub> grass *Themeda triandra* and the C<sub>3</sub> grass *Austrodanthonia caespitosa* (nomenclature follows Buchanan, 1999). While several species of herbaceous dicots are common, N fixing forbs, including *Trifolium subterraneum* and *T. striatum*, are rare and form an extremely small fraction (<0.01%) of the biomass. The community also contains the N-fixing woody twining species *Bossiaea prostrata*, but

again this species forms only a small fraction of the total biomass (~1%). The soil at the site is a black vertisol formed of basaltic clay, pH ~6, with low total N (~0.2%) and extractable P (~20 mg kg<sup>-1</sup>). Site mean annual temperature is 11.6 °C and rainfall occurs mainly during the cooler months of April to October, with a mean annual rainfall of 560 mm, although in the 5 years since the experiment commenced the mean annual rainfall has been 322 mm, ranging from 223 to 457 mm.

### Assessment of soil available nutrient levels

The temperate perennial grasslands of Tasmanian lowlands are exposed to lengthy annual droughts with reliable rainfall occurring only between May and October. Thus, plant growth and soil microbial activity is limited to periods during which the soil is both sufficiently moist and warm, namely the spring months of approximately August to November. Therefore, we estimated soil N availability during the spring months each year from 2004 to 2006 as sampling during this period provided the most ecologically relevant estimate of soil N availability since plants are dormant for the rest of the year. Biological N transformations are also unlikely to occur at other times of the year as microbial activity would be limited by dry soils and low temperatures.

Soil N availability was measured using ion exchange membranes (Cain *et al.*, 1999; Bowatte *et al.*, 2007). As ion exchange membranes interact with the soil solution, they act as artificial roots and provide a measure of plant available N rather than the actual soil N concentration (Cain *et al.*, 1999; Bowatte *et al.*, 2007). DON in the soil consists of both high and low MW fractions, with the low MW DON being predominantly composed of amino acids (Jones *et al.*, 2005). Because it is only the low MW DON that most plant species can access, Jones *et al.* (2005) recommend that comparisons should only be made between this pool and the inorganic pool when appraising the amount of plant available N. In addition to readily binding both nitrate and ammonium ions, ion exchange resins are effective at competing for low MW DON and thus provide an appropriate indication of plant-available DON (Skogley & Dobermann, 1996; Langlois *et al.*, 2003).

Membrane sheets (50 mm × 10 mm, VWR International Ltd, Poole, UK) were fixed to plastic plant labels (100 mm × 15 mm) to make a membrane soil stick. Ten membrane soil sticks were inserted in the soil in random locations in each plot during maximum spring growth in 2004, 2005 and 2006. Sticks were pushed into the soil until the top of the sheet was at the soil surface, making the effective depth sampled 50 mm; 81 ± 10% of roots occur in this region. Membrane sticks were

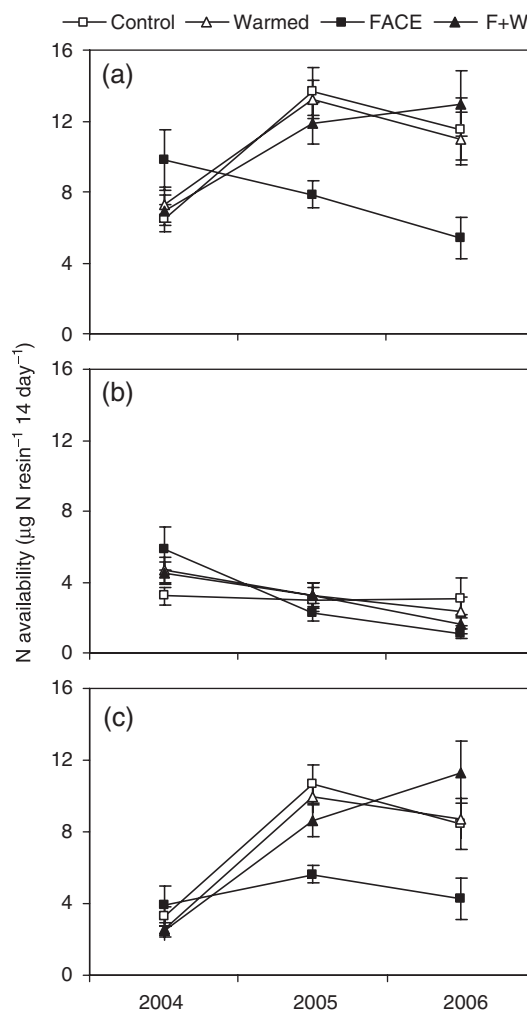
deployed twice during the spring in each year, with the exact timing depending upon rainfall and temperature patterns in order to assess N availability during times of maximum plant growth. On each occasion, the sticks were removed after 14 days. After removal, membranes were washed with distilled water, extracted with 25 mL of 0.05 M HCl and analysed for nitrate, ammonium and total soluble N using an FIAstar 5000 flow injection analyser (Foss Tecator AB, Hoeganaes, Sweden). DON was determined as the difference between total soluble N and mineral N.

### Statistical analyses

Data were analysed by repeated measures mixed model analysis of variance (ANOVA) using general linear model procedures in the SAS statistical software package, version 9.1 (*SAS/STAT User's Guide*, version 9.1, SAS Institute Inc., 2003, Cary, NC, USA). The 20 estimates of soil N availability for each spring, being two samples with each of 10 ion exchange sticks per spring, were pooled to provide a single estimate of soil N availability for each plot in each spring. Proportional data were transformed using the arc-sine transformation before analysis to stabilize variances (Quinn & Keough, 2002). The Ryan–Einot–Gabriel–Welsch *post hoc* comparison was used where ANOVA indicated there were significant treatment effects (Day & Quinn, 1989).

### Results

When soil N availability was investigated during years 3–5 of the TasFACE experiment (2004–2006), we found that for total available soil N, there was a significant  $\text{CO}_2 \times \text{Warming} \times \text{Time}$  interaction ( $P < 0.001$ ), indicating that both warming and  $\text{CO}_2$  treatment affected the temporal response of total available soil N. This was due to the fact that total available soil N was significantly lower in unwarmed FACE plots than in all others in both 2005 ( $P < 0.001$ ) and 2006 ( $P < 0.005$ ; Fig. 1a). Soluble N in the soil solution consists of both mineral ions and DON (Robertson & Groffman, 2007). DON formed a substantial component of the soluble pool of soil N during spring 2004, accounting for  $62.4 \pm 2.1\%$  (mean  $\pm$  SE throughout) of total available soil N. By spring 2005, however, the DON fraction accounted for only  $21.2 \pm 2.6\%$  of total available soil N and declined further, to  $14.4 \pm 1.6\%$  in spring 2006. There were no significant treatment effects on soil DON availability (Fig. 1b), or on the relative proportion of total available soil N that was DON. Thus, while the availability of DON differed among years, there was no clear impact of either elevated  $\text{CO}_2$  or warming (Fig. 1a).



**Fig. 1** Soil total nitrogen (a), dissolved organic nitrogen (b) and mineral nitrogen (c) availability in the TasFACE experiment during spring from 2004 to 2006 (mean  $\pm$  SE,  $n = 3$ ). Treatments shown are unwarmed (squares) and warmed (triangles) plots maintained at current (empty symbols) or elevated  $\text{CO}_2$  (filled symbols).

Because neither elevated  $\text{CO}_2$  nor warming affected soil DON availability in any year, the significant treatment effects on total available soil N in 2005 and 2006 were due to variation among treatments in the mineral N fraction in the soil solution (Fig. 1c). In 2004, the availability of mineral N in the soil was similar in all experimental plots ( $P = 0.15$ ; Fig. 1c). In spring 2005, however, the availability mineral N was 47.3% lower in unwarmed FACE plots than in the control plots ( $P < 0.001$ ) and in spring 2006, this difference was 49.6% ( $P < 0.004$ ; Fig. 1c). It appears that the unwarmed FACE treatment prevented the substantial increase in mineral N availability that occurred between spring 2004 and spring 2005 in all other treatments (Fig. 1c).

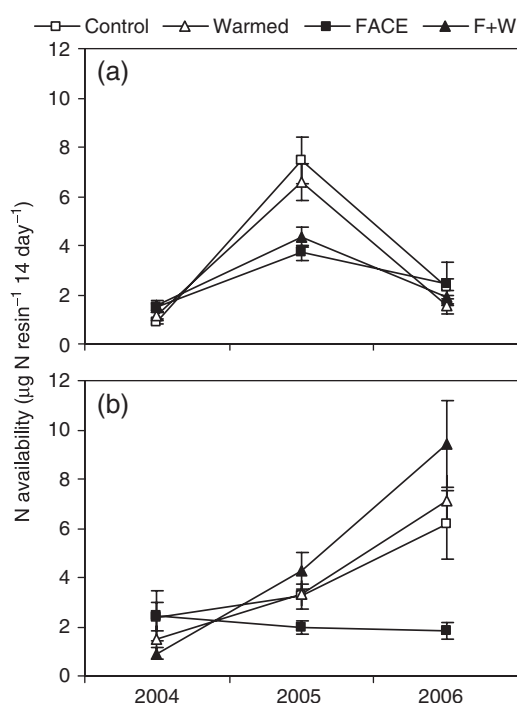
Thus, the substantial decline in the availability of total N in the unwarmed FACE plots (Fig. 1a) was due to a reduction in mineral N (Fig. 1c).

Soil organic N is transformed from polymers, to monomers, to mineral form in strict sequence (Schimel & Bennet, 2004). Organic N is first converted to ammonium, which is then converted to nitrite, which rapidly oxidizes to nitrate in aerobic soils (Schimel & Bennet, 2004). In 2004, there were no significant treatment effects on the total mineral N availability ( $P = 0.41$ ; Fig. 1c), ammonium availability ( $P = 0.55$ ; Fig. 2a) or nitrate availability ( $P = 0.28$ ; Fig. 2b). However, significant differences among treatments in both ammonium ( $P < 0.001$ ) and nitrate ( $P < 0.03$ ) availability had developed by spring 2005 (Fig. 2). Ammonium availability was significantly lower in both warmed and unwarmed FACE plots than it was in either warmed or unwarmed control plots in spring 2005 (Fig. 2a), but these differences had disappeared by spring 2006. It appears that during 2005, in which spring rainfall and soil water potential were substantially higher than in either 2004 or 2006 (Table 1), elevated CO<sub>2</sub> reduced the extent of a substantial increase in soil ammonium availability. Further, the elevated CO<sub>2</sub>-induced reduction in soil ammonium availability during 2005 occurred whether

the plots were warmed or not (Fig. 2a). In contrast, warmed and unwarmed FACE plots differed significantly in nitrate availability during 2005 ( $P < 0.03$ ; Fig. 2b), with warmed FACE plots having more than double the nitrate availability of unwarmed FACE plots. Indeed, warmed FACE plots had the highest nitrate availability of any treatment (Fig. 2b). Contrary to the situation for ammonium, in which availability declined between spring 2005 and spring 2006, nitrate availability increased over same period in all plots other than unwarmed FACE plots, in which nitrate availability remained largely unchanged (Fig. 2c). Thus, the substantial reduction in mineral N availability observed between 2004 and 2006 in unwarmed FACE plots was primarily due to a reduction in the availability of nitrate (Fig. 2b).

## Discussion

These results demonstrate that exposure of an unfertilized, perennial pasture to an elevated [CO<sub>2</sub>] of 550  $\mu\text{mol mol}^{-1}$  without warming reduced the availability of soil N by year 4 of treatment and this reduction was greater in year 5. No reduction of any soil N fraction was evident in the third year of the experiment. This delayed effect of elevated CO<sub>2</sub> on soil N availability supports existing theories of how elevated CO<sub>2</sub> might lead to PNL of terrestrial ecosystem productivity (Luo *et al.*, 2004). While elevated CO<sub>2</sub> effects on soil N have been theorized (Luo *et al.*, 2004) and demonstrated (Hungate *et al.*, 2006; Reich *et al.*, 2006a,c) before, we have shown that the addition of canopy warming by 2 °C to the elevated CO<sub>2</sub> treatment completely prevented the elevated CO<sub>2</sub>-induced reduction in soil N availability. Previous work has generally shown that elevated CO<sub>2</sub> increases ecosystem net primary productivity (Norby *et al.*, 2005). It is this increase in primary productivity that leads to the reduction in soil available N, by increasing root uptake of N thereby increasing N immobilization in plant biomass and long-lived soil organic matter (Luo *et al.*, 2004). An increased plant sink for available N at elevated CO<sub>2</sub> is unlikely in this experiment as elevated CO<sub>2</sub> did not increase biomass production in any year (data not shown). Therefore, it is likely that the decline in available N observed in unwarmed FACE plots involved other mechanisms, such as changes in microbial community size, structure or function (Hu *et al.*, 2006). It is possible that the reduction in available N seen in this experiment may have been more severe had elevated CO<sub>2</sub> increased ecosystem net primary productivity. Therefore, while warming prevented the FACE-induced reduction in available N in this experiment, perhaps by increasing N mineralization, this might not be sufficient to balance a reduction



**Fig. 2** Availability of ammonium (a) and nitrate (b) in the soil in the TasFACE experiment during spring from 2004 to 2006 (mean  $\pm$  SE,  $n = 3$ ). Treatments shown are unwarmed (squares) and warmed (triangles) plots maintained at current (empty symbols) or elevated CO<sub>2</sub> (filled symbols).

**Table 1** Effects of CO<sub>2</sub> and warming treatments on mean soil water potential  $\pm$ SE (kPa) during spring from 2004 to 2006 in the TasFACE experiment

CO <sub>2</sub> warming	Control		FACE	
	Unwarmed	Warmed	Unwarmed	Warmed
2004	-97.2 $\pm$ 17.4	-104.5 $\pm$ 21.5	-74.5 $\pm$ 12.0	-115.0 $\pm$ 11.6
2005	-29.4 $\pm$ 5.8	-41.2 $\pm$ 8.1	-21.0 $\pm$ 3.0	-42.7 $\pm$ 11.3
2006	-164.4 $\pm$ 34.5	-132.1 $\pm$ 33.2	-148.0 $\pm$ 17.3	-167.9 $\pm$ 8.4

CO<sub>2</sub>, carbon dioxide; FACE, free air CO<sub>2</sub>-enrichment.

in available N in ecosystems where elevated CO<sub>2</sub> does stimulate primary productivity. Indeed, it is entirely possible that the reduction in available N prevented any stimulation of productivity by FACE treatment in years 4 and 5. Therefore, while warming prevented elevated CO<sub>2</sub> from reducing available N in this experiment, similar results might not be obtained in other systems, particularly those in which elevated CO<sub>2</sub> has been demonstrated to enhance primary productivity (Reich *et al.*, 2006b,c). Clearly, there is a need for more experimental investigation of the interactive effects of elevated CO<sub>2</sub> and warming on N dynamics in other ecosystems in order to determine whether the results seen here are applicable beyond this low productivity grassland.

Strong sensitivity of soil N transformations to temperature has been demonstrated previously (Castaldi, 2000; Fenner *et al.*, 2006). In arable soils, for example, denitrification was found to have a  $Q_{10}$  value of 8.9 while other transformation rates all had  $Q_{10}$  values of approximately 2 (Castaldi, 2000). Thus, increasing soil temperature by an average of 0.8 °C, as occurs in the TasFACE experiment, would increase denitrification rates by over 70% based on a  $Q_{10}$  of 8.9 (Castaldi, 2000). Therefore, warming the soil by even <1 °C has the potential to substantially alter the balance of N transformations, which would be likely to alter soil N availability. However, the results from this study show that this level of warming only altered N availability at elevated CO<sub>2</sub> concentrations. While the exact mechanism whereby warming prevents the reduction of available soil N by elevated CO<sub>2</sub> is unknown, these results do provide testable hypotheses.

The interaction of elevated CO<sub>2</sub> and warming on available N suggests (1) that the decrease in N availability driven by elevated CO<sub>2</sub> occurs within pools that are temperature sensitive, or (2) warming alters the C allocation responses of plants to elevated CO<sub>2</sub> such that the conditions driving the reduction in available N do not develop. The decrease in soil ammonium availability in elevated CO<sub>2</sub> plots in 2005 indicates that either

ammonification rates were significantly depressed by elevated CO<sub>2</sub> or ammonium uptake, immobilization or gaseous loss was significantly increased. Increased microbial immobilization of N in response to elevated CO<sub>2</sub> has been demonstrated in several systems (de Graaff *et al.*, 2006). Meta-analysis has shown that elevated CO<sub>2</sub> treatment results in an average increase in soil C of 1.2% per annum and is associated with an increase in microbial activity of 17.7% (de Graaff *et al.*, 2006). This suggests that soil microorganisms are generally C-limited, so 'priming' soils by increasing soil C inputs under elevated CO<sub>2</sub> will substantially enhance microbial immobilization of N (de Graaff *et al.*, 2006). This is particularly important in the context of the results shown here, as the warming treatment affected the CO<sub>2</sub>-response of plant biomass allocation. In this experiment, plants grown in unwarmed elevated CO<sub>2</sub> plots allocated significantly more biomass to the roots than plants grown in warmed plots at elevated CO<sub>2</sub> (data not shown). Thus, it is possible that the CO<sub>2</sub>-priming effect, discussed above, only occurs in the absence of warming in this experiment, explaining the interactive effects of elevated CO<sub>2</sub> and warming on soil N availability. Thus, the results shown here may be due to treatment effects on plant behavior influencing microbially mediated processes.

Treatment effects on soil C inputs are also likely to affect the fate of ammonium, which might lead to alterations in nitrate production and availability. Elevated nitrification rates following trenching in a New Zealand pine plantation suggest that reducing C inputs by roots diminishes heterotrophic ammonium assimilation, shifting the competitive balance in favour of nitrifiers (Ross *et al.*, 2001). Increased plant C inputs to soils under elevated CO<sub>2</sub> in the absence of warming may also affect the fate of ammonium by increasing heterotrophic ammonium uptake (Hungate *et al.*, 1997, 1999; Mikan *et al.*, 2000) and decreasing nitrification rates (Hungate *et al.*, 1997, 1999), thus leading to a decline in soil nitrate. It is unlikely that soil moisture differences contributed to the interaction between

elevated CO<sub>2</sub> and warming effects as soil moisture in unwarmed FACE and unwarmed control plots did not differ significantly (Table 1).

In conclusion, our study shows that soil N availability in a temperate pasture declined over time upon exposure to elevated CO<sub>2</sub>, but importantly it also shows that the addition of a 2 °C warming treatment nullified this trend. It is likely that this result was produced by interactions between plant C allocation responses and the microbial community. Thus, since global temperatures are expected to increase along with the increasing atmospheric concentration of CO<sub>2</sub>, it is possible that elevated CO<sub>2</sub> will not always reduce soil N availability, as was the case in this ecosystem where plant productivity, and hence probably the plant sink for N was not stimulated by CO<sub>2</sub> fumigation. It remains unknown whether PNL could be overcome by warming-induced increases in N availability where the plant demand for N is also significantly stimulated by CO<sub>2</sub> fertilization. This has major ramifications for future ecosystem productivity and C sequestration potential of terrestrial ecosystems.

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