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REGULAR ARTICLE

Warming has a larger and more persistent effect than elevated CO_2 on growing season soil nitrogen availability in a species-rich grassland

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Abstract
Background and aims The terrestrial biosphere's ability to capture carbon is dependent upon soil nitrogen (N) availability, which might reduce as CO_2 increases, but global warming has the potential to offset CO_2 effects. Here we examine the interactive impact of elevated CO_2 (eCO_2) and warming on soil N availability and transformations in a low-fertility native grassland in Tasmania, Australia.
Methods Using ion exchange membranes, we examined soil nitrogen availability during the growing season from 2004 to 2010 in the TasFACE experiment. We also estimated soil N transformation rates using laboratory incubations.
Results Soil N availability varied strongly over time but was more than doubled by experimental warming of 2°C, an impact that was consistent from the fifth year of the experiment to its conclusion. Elevated CO_2 reduced soil N availability by ~28%, although this varied strongly over time. Treatment effects on potential N mineralisation also varied strongly from year to year but tended to be reduced by eCO_2 and increased by warming.
Conclusions These results suggest that warming should increase soil N availability more strongly than it is suppressed by eCO_2 in low fertility grasslands such as this, stimulating terrestrial carbon sinks by preventing eCO_2-induced nitrogen limitation of primary productivity.

Keywords Biogeochemistry · Face · Nutrient availability · Nitrate · Temperate grassland · Warming experiment

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Introduction

Since anthropogenic emissions of CO_2 continue to rise, it is critical that we determine whether carbon sink strength will saturate at a particular atmospheric concentration of CO_2, as other factors become limiting (Canadell et al. 2008; Pepper et al. 2005). Nitrogen (N) is the mineral nutrient most widely limiting to plant growth and hence to productivity in terrestrial ecosystems (Melillo et al. 1993; Vitousek and Howarth 1991), so that it is often proposed as the primary controller of the biomass response to elevated CO_2 concentrations (Finzi et al. 2006; Luo et al. 2004; McMurtrie et al. 2008; Reich et al. 2006b; Ross et al. 1996; Zak et al. 2003). Most N in the terrestrial biosphere is absorbed from the soil as nitrate, ammonium and low molecular weight organic molecules such as amino acids supplied by the decomposition of organic matter (Hofmockel et al. 2011; Hofmockel et al. 2007; Schimel and Bennett 2004). It is evident from both modelling and experimental investigations that N limitation could prevent ecosystem productivity from responding to the strong fertilization effect of rising atmospheric CO_2 (Reich and Hobbie 2013; Reich et al. 2006a). Moreover, elevated CO_2 (eCO_2) alters resource availability to soil microbes (Hu et al. 1999), which might cause a decline in ecosystem N availability through a process termed progressive N limitation (Luo et al. 2004). In essence, the rising CO_2 concentration stimulates photosynthesis, increasing plant nutrient demand thereby increasing the proportion of N in the ecosystem that is immobilized in biomass (Luo et al. 2004). Changes in the concentrations of nutrients (Aerts 1997), secondary metabolites (Carrera et al. 2005) or structural components (Moore et al. 1999) in deposited litter could also alter relative rates of mineralization and immobilization. Exudation of C compounds into the soil by plants also increases as CO_2 rises (Norby et al. 1987; Uselman et al. 2000), stimulating soil heterotrophs and immobilising N in microbial biomass (de de Graaff et al. 2007), but potentially also increasing microbial mobilisation of N from longer-lived soil organic matter pools (i.e. priming; Bengtson et al. 2012; Drake et al. 2011). It is also possible that root exudations or alteration of soil conditions in response to eCO_2 could affect the composition or activity of the soil microbial community (Osanai et al. 2015). These changes in biogeochemistry could feedback on plant protein contents and growth rates (Hu et al. 1999). Over time, therefore, it is possible that the rising CO_2 concentration in the atmosphere will actually reduce productivity in terrestrial ecosystems (Hu et al. 1999; Luo et al. 2004; Reich et al. 2006a).

The influence of warming on organic matter decomposition and N mineralization are well-studied (Chung et al. 2013; Gholz et al. 2000; Rustad et al. 2001), but there has been little work examining how increasing temperature will interact with increasing CO_2 concentration to influence soil N availability in the field (Dijkstra et al. 2010; Hovenden et al. 2008a; Larsen et al. 2011). The decomposition of organic matter releases organic N into the soil solution as dissolved organic N (DON), which is oxidised to ammonium via ammonification, which is in turn oxidised to nitrate via nitrification, with the net conversion of organic N to inorganic N termed N mineralization. In most ecosystems, increasing the temperature will increase rates of litter decomposition and N mineralization, so it is possible that this response will reduce or even reverse the impacts of rising CO_2 concentration on N availability (Parton et al. 2007). The few studies that have specifically examined the interaction between eCO_2 and warming on N availability have given varying results such that a meta-analysis by Dieleman et al. (2012) concluded that the positive effects of warming tended to balance the negative effects of eCO_2 leading to no net change in N availability in warmer, higher CO_2 conditions. This has also been supported by more recent experimental results (Bjorsne et al. 2014). However, major uncertainties relate to the relative time scales of plant and microbial community responses to warming and eCO_2 (Reich et al. 2006b; Reich et al. 2004). If these treatment effects resolve over different time-scales then short-term experimental results, even those collected over several years, might not indicate the true magnitude of treatment effects.

A further complication lies in the fact that productivity responses of terrestrial ecosystems to global changes might be co-limited by several different factors. The idea of multiple resource-limitation is that as the availability of any one limiting factor increases, the availability of other factors becomes increasingly limiting to production (Reich and Hobbie 2013; Reich et al. 2014). This interplay between different factors can explain why various systems respond differently or in unpredictable ways to eCO_2 but is also one reason that other climate changes might interact with eCO_2 in a non-additive manner (Dieleman et al. 2012). For instance, the effects of eCO_2 on N-cycling are strongly dependent upon N supply (Reich and Hobbie 2013) but one of the major implications of multiple resource limitation theory is that warming might substantially alter the way that the rising [CO_2] will affect ecosystem productivity by changing which factors are limiting and to what degree. Organic matter decomposition rates increase with increasing temperature, so warming will increase the rate of mineralisation of a range of nutrients (Ma et al. 2011), potentially increasing the availability of growth-limiting nutrients and thereby influencing the degree of co-limitation of productivity responses to eCO_2. Such information is crucial if terrestrial ecosystem function and C sink strength are to be predicted (Luo et al. 2011; Reich et al. 2006b), so there is a continued need for results from experiments that manipulate other factors in addition to [CO_2].

We sought to determine if experimental warming interacted with an increase in CO_2 concentration to influence soil N availability and N-mineralisation in plots of native grassland in southeastern Tasmania, Australia and to test whether any treatment-induced changes were sustained over time. In this system, the availability of N is low as are N-fixation rates, therefore there is considerable potential for warming to interact with the eCO_2 effect on N availability. Specifically, we test the following hypotheses: 1. the reduction in soil N availability caused by eCO_2 is sustained in the longer-term; 2. warming increases soil N availability; and 3. treatment-induced changes in soil N availability are underlain by changes in potential N mineralisation rates.

Methods

Study site and experimental design

The TasFACE Climate Change Facility was established in an area of native lowland temperate grassland on a low fertility basalt plain in south-eastern Tasmania, Australia (42 ° 42′S, 147 ° 16′E). The region has a modified Mediterranean climate characterised by mild moist winters and warm dry summers. Total precipitation is low (~400 mm per annum) and potential evaporation is high (~1250 mm per annum), so there is significant summer drought. The grassland community at the site is species-rich with 51 vascular plant species recorded in the experimental plots. The vegetation is dominated by the perennial grasses *Themeda triandra* Forssk. (the only C_4 species), *Rytidosperma caespitosum* (Gaudich.) Connor & Edgar and *Rytidosperma carphoides* (F.Muell. ex Benth.) Connor & Edgar, although almost one-third of the recorded species are native perennial forbs. The full species list is provided elsewhere (Hovenden et al. 2006).

The experiment consisted of 12 Free Air CO_2 Enrichment (FACE) rings of 1.5 m diameter, in which vegetation was exposed to either ambient or elevated CO_2, and were either warmed or unwarmed. Thus, the experiment was a factorial 2 × 2 design with three replicate plots of each CO_2 × warming combination, viz. unwarmed control, warmed control, unwarmed FACE and warmed FACE. FACE rings were fumigated to 550 μmol mol^{-1} by the FACE method, using a modified pure-CO_2 injection system (Hovenden et al. 2006; Miglietta et al. 2001). Warming was provided by the addition of 140 W m^{-2} of infrared radiation (IR) using 240 V 250 W Emerson Solid Ceramic Infrared Emitters suspended 1.2 m above the soil surface above the centre of each ring. The IR emitters operated continuously and provided an average warming of canopy temperature of 1.98°C and of soil temperature at 1 cm depth by 0.82°C over the year. The warming treatment also reduced relative humidity by an average of 5.1% over the same period. Warming treatment performance was monitored continuously and lamps replaced as they aged to maintain the treatment effects. Full details of experimental design and system performance were provided elsewhere (Hovenden et al. 2006; Hovenden et al. 2008b; Hovenden et al. 2017; Hovenden et al. 2008c). Rainfall and soil moisture conditions are provided in Supplementary Table S1 and Fig. S1.

Soil nutrient analyses

Available soil nitrogen was assessed each spring, the time of maximum plant growth and microbial activity in this ecosystem, using ion exchange membranes as previously described (Hovenden et al. 2008a). As ion exchange membranes interact with the soil solution, they act as artificial roots and provide a measure plant available N rather than the actual soil N concentration (Cain et al. 1999, Bowatte et al. 2007). Dissolved organic N (DON) in the soil consists of both high and low molecular weight fractions, with the low weight fraction consisting predominantly of amino acids (Jones et al. 2005). Since it is only the low weight DON that most plant species can access, this is the organic N pool most important for assessments of overall availability of N in the soil solution. Ion exchange resins compete effectively for low molecular weight DON and therefore provide a useful indication of plant-available organic N (Skogley and Dobermann 1996, Langlois et al. 2003). Ten ion-exchange membrane sticks bearing both an anion and cation exchange resin were deployed per plot each spring from 2004 to 2010. Resin strips were deployed in either late September or early October, with the exact timing depending upon rainfall and temperature patterns but the timing did not vary by more than 2 weeks over the entire 7 year period. Sticks were pushed into the soil until the top of the sheet was at the soil surface, making the effective depth sampled 50 mm; at this site 81 ± 10% of roots occur in this depth region. In each year, sticks were placed in the soil for a 14 day period and replaced immediately with a second set of sticks, meaning that N availability was assessed for the a 4 week period each spring. After removal, membranes were washed with distilled water to remove adhering soil, then the resin membranes were extracted with 25 ml of 0.05 M HCl with shaking for 12 h. Nitrate, ammonium and total soluble N in the resultant extract was measured using an FIAstar 5000 flow injection analyser, (Foss Tecator AB, Hoeganaes, Sweden). Dissolved organic N (DON) was calculated as the difference between total soluble N and mineral N, the sum of ammonium-N and nitrate-N.

Potential net nitrogen mineralization was determined annually using soils sampled from each plot during spring in 2007 to 2010. Four 2.5 cm diameter soil cores 5 cm in depth were collected from each plot at a time corresponding with peak growth. The four soil cores were composited in the field, stored on ice and then returned to the laboratory where soil was sieved to 4 mm and visible roots removed by hand. Initial extractable N was determined by flow injection analysis (as above) following extraction in 100 mL 2 M KCl of a 10 g subsample. A second 10 g sub-sample was incubated at 23°C for 28 d, then final extractable N concentration determined as above. Net ammonification, nitrification and mineralization were calculated as the difference in ammonium, nitrate and mineral N concentrations of pre- and post- incubated soils.

Statistical analysis

Soil available nutrient levels and potential net N mineralisation rates were analysed by a repeated measures two-factor analysis of variance in R (R Development Core Team 2016), with [CO_2] and warming the two fixed factors. All data were checked for normality and variance heteroscedasticity and data were transformed where necessary using the Box-Cox transformation in the *MASS* R package (Venables and Ripley 2002). Where ANOVA indicated there were significant treatment effects, means were compared using Tukey's Honestly Significant Difference test (Logan 2010).

Results

Nitrogen availability

There were significant impacts of both warming and eCO_2 on soil N availability but these impacts tended to vary among years for most measures. Over the 7 year measurement period, total available N (i.e. nitrate + ammonium + low molecular weight organic N) during the period of peak growth was 15.9 ± 2.6 μg N resin^{-1} fortnight^{-1} in plots exposed to eCO_2 and 21.7 ± 4.5 μg resin^{-1} fortnight^{-1} in control plots ($F_{1,8}$ = 3.49, P = 0.099) but was 139% higher in warmed plots (26.4 ± 4.7 μg resin^{-1} fortnight^{-1}) than in unwarmed plots (11.3 ± 1.6 μg resin^{-1} fortnight^{-1}; $F_{1,8}$ = 22.0, P = 0.002). There was no significant interaction between eCO_2 and warming on total available N ($F_{1,8}$ = 0.11, P = 0.74). The magnitude and significance of these effects, however, was strongly dependent upon time (Fig. 1), with significant Year × Warming ($F_{6,48}$ = 14.7, P < 0.0001) and Year × CO_2 ($F_{6,48}$ = 3.08, P < 0.01) interactions but no significant three-way interaction ($F_{6,48}$ = 0.96, P = 0.44). Thus, both the warming and the eCO_2 effect on total N availability depended strongly upon year but the treatment effects remained independent (Fig. 1). The variation among years and plots in soil N availability was not related to differences in soil moisture conditions (Fig. S2, S3).

Treatment effects were most pronounced on the availability of nitrate (Fig. 1c) with smaller and less reliable impacts of both warming and eCO_2 on the availability of either dissolved organic N (DON; Fig. 1a) or ammonium (Fig. 1b). While both DON and ammonium availability varied over time ($F_{6,48}$ = 10.1, P < 0.0001 for DON; $F_{6,48}$ = 10.2, P < 0.0001 for ammonium), there was no general trend in the effect of time nor in the effect of experimental treatments (Fig. 1a, b). Nitrate availability, in contrast, tended to rise substantially from 2004 to 2008, then decline sharply from 2008 to 2010 (Fig. 1c). Since nitrate became the dominant N form in TasFACE soils over this period, total available N followed the same general trajectory (Fig. 1d). Treatment effects on nitrate and total available N similarly became more pronounced over time.

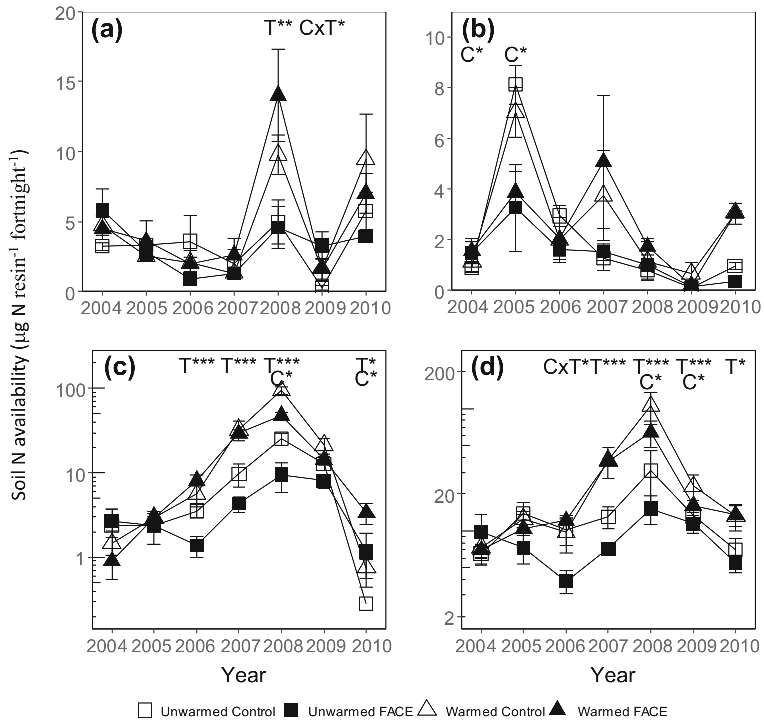

Fig. 1 Spring soil nitrogen availability over a 7 year period in the TasFACE experiment. **a** Dissolved organic N, **b** ammonium-N, **c** nitrate-N and **d** total available N. Values are means ± SEM ($n = 3$). Letters and asterisks indicate significant treatment effects in a particular year obtained from 2-factor analysis of variance and after correction for false discovery: T, temperature effect; C, CO_2 effect; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Warming increased soil nitrate availability from 2006 to 2008 (Fig. 1c) and total available N from 2007 to 2010 (Fig. 1d).

While eCO_2 tended to reduce both nitrate and total N availability, this effect was only significant in 2008 and 2010 for nitrate and in 2008 and 2009 for total N. However, the combination of the generally depressive effects of eCO_2 and the significantly stimulatory effects of warming on nitrate and total N availability led to the unwarmed FACE plots having the lowest N availability, followed by the unwarmed control plots with the warmed FACE plots having the second highest N availability and the warmed control plots the highest (Fig. 1d). Treatment effect sizes were unrelated to soil moisture conditions (Fig. S3).

Proportions of soil nitrogen forms

The combined impacts of time and experimental treatments on the availability of individual soil N forms resulted in substantial alterations in the proportion of available N existing in those forms (Fig. 2). While temporal trends dominated the proportion of available N present as DON ($F_{6,48} = 59.9$, $P < 0.0001$), ammonium ($F_{6,48} = 39.9$, $P < 0.0001$) and nitrate ($F_{6,48} = 56.4$, $P < 0.0001$), there were strong, significant and consistent impacts of warming on the proportions of DON ($F_{1,8} = 5.5$, $P = 0.05$; Fig. 2a) and nitrate ($F_{1,8} = 6.0$, $P = 0.04$; Fig. 2c) and a significant year × warming interaction effect on the proportion of ammonium ($F_{6,48} = 3.6$, $P = 0.005$; Fig. 2b). Warming reduced the proportion of DON present in the soil from 34.8 ± 3.9 to 28.8 ± 4.0% but increased the proportion of nitrate present from an overall average of 45.8 ± 4.5 to 55.0 ± 5.0%. Thus, the impact of warming was to accelerate the conversion of organic N to nitrate in soils at TasFACE.

Elevated CO_2, in contrast, had no overall impacts on the proportional representation of different N forms but had an impact that tended to vary over time, as evidenced by a significant year × CO_2 interaction on the proportion of DON ($F_{1,8} = 2.5$, $P = 0.03$; Fig. 2a) and of nitrate ($F_{1,8} = 3.1$, $P = 0.01$; Fig. 3b). These effects were only expressed in 2009 and 2010, the final years of the experiment (Fig. 2).

Potential nitrogen transformations

In order to determine whether treatments affected the inherent capacity of the TasFACE soils to convert soil organic matter to mineral forms, we assessed N mineralisation rates in TasFACE soils from 2007 to

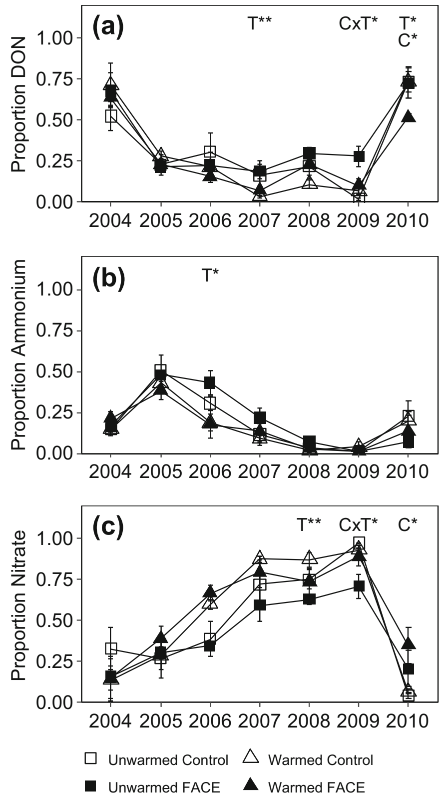

Fig. 2 The proportion of available soil nitrogen present as **a** dissolved organic N (DON), **b** ammonium and **c** nitrate in the TasFACE experiment during peak growing season from 2004 to 2010. Values are means ± SEM, $n = 3$

2010. There was significant variation among years in potential net mineralisation ($F_{1,8} = 23.4$, $P < 0.0001$) as well in potential net ammonification ($F_{1,8} = 4.17$, $P = 0.009$) and net nitrification ($F_{1,8} = 20.9$, $P < 0.0001$). Elevated CO_2 tended to reduce both potential nitrification ($F_{1,8} = 6.11$, $P = 0.04$) and net mineralisation ($F_{1,8} = 6.10$, $P = 0.04$), but these effects also varied over time, as evidenced by significant Year × CO_2 interactions ($F_{3,72} = 5.59$, $P = 0.002$ for nitrification; $F_{3,72} = 5.22$, $P = 0.003$). There was no overall effect of warming on any of the potential N transformation rates but there was significant Year × Warming interactions for both potential nitrification ($F_{3,72} = 3.44$, $P = 0.02$) and for potential N mineralisation ($F_{3,72} = 4.27$, $P = 0.008$). There were no significant three-way interactions. Therefore, the impact of both eCO_2 and warming on potential soil N transformations varied from year to year. Interestingly, experimental treatments influenced potential N transformations significantly in 2008 and 2010 but had no significant effect in either 2007 or 2009 (Fig. 3). However, treatment impacts were similar in 2008 and 2010, with warming increasing potential mineralisation and nitrification while eCO_2 substantially reduced both of these N transformations (Fig. 3). In 2009, the trend was similar to in 2008 and 2010 but treatment effects were slight, whereas in 2007 there were no indications of treatment effects. There was little change in ammonium concentration in any treatment in any year, leading to no net ammonification in these soils. Thus, apart from in 2007, the tendency was for warming to accelerate the conversion of organic to mineral N whereas eCO_2 reduced the conversion. The large differences in mean net N mineralisation rates among the years were unrelated to differences among years in soil moisture or rainfall patterns (Fig. S4).

Discussion

We had previously suggested, based on the 2004–2006 data, that warming interacted with eCO_2 to prevent an eCO_2-driven reduction in soil N availability in the TasFACE experiment (Hovenden et al. 2008a). This longer dataset, however, indicates clearly that the warming treatment acted independently of and dominated the CO_2 treatment so that plots exposed to both 2°C warming and 550 μmol CO_2 mol^{-1} were not just the same as the controls (as in the early years) but had consistently higher nitrogen availability. This difference was maintained from year 6 (i.e. 2007) to the conclusion of the TasFACE experiment after nine full years of treatments (i.e. 2010). These results differ substantially from earlier research which mostly suggested that modest experimental warming tended to negate but not overwhelm the effects of eCO_2 on soil N availability (Bjorsne et al. 2014; Dieleman et al. 2012; Hovenden et al. 2008a) or that the influence of warming was insufficient to offset the eCO_2 effect (Dijkstra et al. 2012). Instead, we found that the impact of eCO_2 was smaller and more variable than the warming effect, agreeing with results from the BioCON that indicated that eCO_2 had smaller and less consistent impacts on soil N pools and fluxes than other factors (Mueller et al. 2013). This is unlikely to be due to the TasFACE ecosystem being unusually responsive to warming, as the acceleration of N mineralisation by warming was well within the range of previous studies (Rustad et al. 2001). While the results from the PHACE experiment in semi-

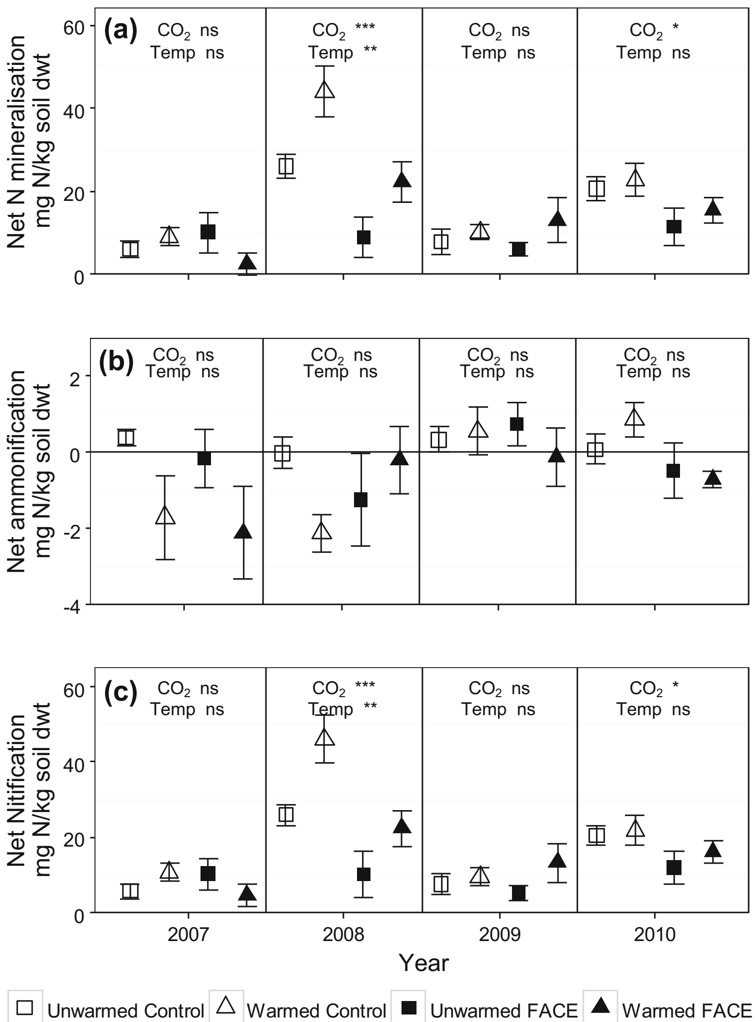

Fig. 3 The impact of elevated CO_2 (CO_2) and warming (Temp.) treatments on **a** nitrogen mineralisation, **b** net ammonification and **c** net nitrification potential of soils collected from the TasFACE experiment during spring 2007 to 2010. Values are means ± SEM, *n* = 3

arid rangeland, the experiment most similar to TasFACE, showed that eCO_2 and warming effects on soil N varied substantially from year to year (Carrillo et al. 2012), there is evidence that warming can also overwhelm the eCO_2-induced reduction in soil nitrate availability at least in some years (Mueller et al. 2016), supporting our results. However, treatment effects in the PHACE experiment were related to soil moisture conditions, with greater impacts occurring in drier years (Mueller et al. 2016), which was not the case at TasFACE (Fig. S3).

Importantly, we found no real indication of any sustained interaction between eCO_2 and warming on either N availability or N mineralisation rates. Thus, it is unlikely that elevated CO_2 increased N availability when N mineralisation was stimulated by warming, as had been suggested elsewhere (Chen et al. 2016; Pastore et al. 2016). In the TasFACE experiment warming and eCO_2 effects on N mineralisation and availability were essentially additive and independent, meaning that the response to the combined treatment could be predicted from the response to either applied in isolation. Unlike the situation in other systems (Chen et al. 2016; Hasegawa et al. 2016; Ross et al. 2013; Rütting and Andresen 2015), we found no evidence that eCO_2 increased N availability or mineralisation in any year, aligning with the situation in the PHACE experiment (Carrillo et al. 2012; Mueller et al. 2016). While variation among replicate plots led to few years in which eCO_2 significantly affected N availability, it was clear that soils in unwarmed FACE plots had the lowest nitrate and total available N concentrations of the four treatment combinations (Fig. 1). The strong variation among years in potential N mineralisation rates is surprising but might indicate a dynamic and variable soil microbial community responding to large interannual variation in rainfall. In the years when treatment effects on N mineralisation did occur, they were consistent and had the same impacts observed in field N availability. Thus, soil from warmed plots had higher potential N mineralisation than soil from unwarmed plots and that from FACE plots had lower potential mineralisation rates than occurred in soil from control plots (Fig. 3), with no evidence of interactions between the two. Further, our estimates of potential N mineralisation were done at a single temperature under standardised laboratory conditions. Hence, the realised N mineralisation rate in the warmed plots is likely to be higher than that observed in the incubations, since the soil temperature in the experiment is higher in warmed plots. Therefore, the in situ estimates of N availability and the laboratory estimations of the soil's ability to convert organic N to mineral forms both indicated that eCO_2 would reduce N availability while warming would increase it. The biggest differences were observed in the production and availability of nitrate. Experiments in forest (Bader et al. 2013) and woodland (Hungate et al. 2014) have demonstrated increased leaching of nitrate in response to eCO_2, which could be caused by changes in hydrology, soil N-transformations or a combination of the two. Our results indicate that only changes in hydrology would lead to increased nitrate leaching in this system, since nitrate production or availability was not increased by eCO_2. Our results do not support an eCO_2-derived increase in N availability, rather supporting the reduction in N:P observed in the PHACE experiment (Dijkstra et al. 2012). However, unlike the situation at PHACE, the combination of warming and eCO_2 in the TasFACE experiment increased N:P via warming-derived changes in N availability (Ma et al. 2011). This is important, because it indicates that a modest increase in temperature, only 2°C in the canopy and 1°C in the soil, could be sufficient to prevent eCO_2-driven reductions in N availability in this system, as also occurred in the PHACE experiment in 2011 and 2012 (Mueller et al. 2016). Importantly, variation among years in the impact of both warming and eCO_2 on soil N availability have been linked to variation in soil moisture conditions (Carrillo et al. 2012; Mueller et al. 2016; Osanai et al. 2017), but this does not appear to be the case at TasFACE, in which the soil N availability and treatment impacts were not related to soil moisture availability (Fig. S2, S3).

The reductions in N availability in response to eCO_2 that occurred in the TasFACE experiment are unlikely to have been driven by altered immobilisation of N into plant biomass and therefore the mechanism believed to be the main driver of progressive N limitation, i.e. eCO_2-induced growth stimulation (Luo et al. 2004), is probably not the primary cause of the reduction in N availability in the FACE plots. Biomass production at TasFACE was only stimulated by eCO_2 in 2006 and 2007 (Hovenden et al. 2014) and was unaffected in the other years during the study period. Certainly, eCO_2 did not lead to an increase in the amount of N sequestered in plant biomass in this experiment (Hovenden et al. 2014). Had eCO_2 led to a more consistent stimulation of growth at TasFACE, N availability might have been reduced further and more consistently and in such a situation warming might not have increased N availability in eCO_2 plots to as great an extent. This situation, then, would be more akin to that observed in the PHACE experiment (Dijkstra et al. 2010), in which eCO_2 had a greater impact than warming on N availability. The increases in N availability in the warmed plots are also unlikely to be due to reductions in plant demand for N, since biomass production was largely unaffected by warming in the TasFACE experiment (data not shown). Therefore, we believe that the treatment-induced alterations in N availability in this experiment were not driven by changes in plant uptake.

This finding is consistent with the explanation for PNL in grassland described in Newton et al. (2010). Unlike forests that have a woody biomass pool providing long-term N storage that encourages PNL to occur, grassland plants are often ephemeral and long-term N storage is in the soil rather than the plant. This means that PNL is driven by soil processes that themselves are frequently dependent on microbial activity and consequently PNL effects have the potential to vary spatially and temporally.

In the TasFACE experiment, the reduction in soil N was likely a result of reduced rates of N mineralisation. Both warming and eCO_2 altered the C-to-N ratio of above and below ground material at TasFACE (Osanai et al. 2015; Pendall et al. 2011), with the changes corresponding to the differences in N mineralisation rates observed here. This, coupled with treatment-induced alterations of the soil microbial community composition and function (Hayden et al. 2012; Osanai et al. 2015) are the most likely drivers of the alterations in N availability at TasFACE. Since changes to litter quality (Franck et al. 1997; Gahrooee 1998; Hattenschwiler et al. 1999; Hirschel et al. 1997; Kasurinen et al. 2007; Kemp et al. 1994) and microbial community function (Carney et al. 2007; Drigo et al. 2008; Ebersberger et al. 2004; Hayden et al. 2012; Osanai et al. 2015; Pinay et al. 2007) are widespread in global change experiments, we contend that these changes alone are likely to drive future alterations to nutrient cycling in grasslands, with potential consequences for ecosystem productivity.

Our results also demonstrate that it takes several years before treatment effects on biogeochemical processes are realised, possibly because changes in the quality of organic matter entering the soil will take some time to alter the overall nature of the soil organic matter pool unless inputs are great in comparison to the existing pool size. The reasons that warming effects were not evident during the first four to 5 years of the experiment remain uncertain but a threshold might have been reached after 5 or 6 years of cumulative additions of lower C-to-N tissues that induced a shift from immobilization to mineralization in the warmed plots. It is also likely that treatment effects on the soil environment play a role in the delay. Since potential N mineralisation in the warmed FACE plots was essentially the same as in the unwarmed control plots, due to similarity in litter C-to-N ratio, the differences in soil N availability in the field are likely to be due to treatment effects on the environment, potentially on both biotic and abiotic components. The warming treatment increased soil temperature by approximately 1°C (Hovenden et al. 2006), which would partially account for the increased N availability as warming accelerates litter decomposition (van van Meeteren et al. 2008). However, experimental warming also reduces soil moisture content, particularly during the wet winter and spring months (Hovenden et al. 2008b), which would increase aeration thereby increasing nitrification and reducing denitrification, which is an anaerobic process and most likely to occur during moist periods (Bollmann and Conrad 1998). In an American prairie, warming reduced soil moisture and productivity also after a substantial lag period (Sherry et al. 2008). The treatments at TasFACE were also likely to influence plant behaviour, especially root exudation, altered levels of which would have influenced microbial activity and immobilization/mineralization rates (Bais et al. 2006; Bardgett et al. 1999; Broeckling et al. 2008; Kuzyakov et al. 2007; Phillips et al. 2009), thereby contributing to differences between field N availability and laboratory mineralization rates.

These results demonstrate that eCO_2 can lead to a reduction in soil N availability without the need for increased sequestration of N into plant biomass but that warming of just 2°C at the soil surface overwhelms this reduction. Therefore, N availability in systems in which the stimulation of biomass by eCO_2 is slight might actually have increased N availability in the future, driven by acceleration of N mineralisation rates. Since the acceleration of decomposition and therefore nutrient cycling is likely to be tightly dependent upon the degree of warming, future nutrient availability and therefore ecosystem productivity will be highly dependent upon just how much warming accompanies the rise in CO_2 concentration.

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