Soil nitrogen cycle unresponsive to decadal long climate change in a Tasmanian grassland

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Abstract Increases in atmospheric carbon dioxide (CO₂) and global air temperature affect all terrestrial ecosystems and often lead to enhanced ecosystem productivity, which in turn dampens the rise in atmospheric CO₂ by removing CO₂ from the atmosphere. As most terrestrial ecosystems are limited in their productivity by the availability of nitrogen (N), there is concern about the persistence of this terrestrial carbon sink, as these ecosystems might develop a progressive N limitation (PNL). An increase in the gross soil N turnover may alleviate PNL, as more mineral N is made available for plant uptake. So far, climate change experiments have mainly manipulated one climatic factor only, but there is evidence that single-factor experiments usually overestimate the effects of climate change on terrestrial ecosystems. In this study, we investigated how simultaneous,

decadal-long increases in CO₂ and temperature affect the soil gross N dynamics in a native Tasmanian grassland under C3 and C4 vegetation. Our laboratory ¹⁵N labeling experiment showed that average gross N mineralization ranged from 4.9 to 11.3 μg N g⁻¹ day⁻¹ across the treatment combinations, while gross nitrification was about ten-times lower. Considering all treatment combinations, no significant effect of climatic treatments or vegetation type (C3 versus C4 grasses) on soil N cycling was observed.

Keywords Elevated CO₂ · Warming · Gross mineralization · Gross nitrification · Nutrient limitation

Introduction

The climate is changing globally, mainly due to anthropogenic emissions of carbon dioxide (CO₂), which affects all ecosystems (IPCC 2019). The specific ecosystem responses to these changes depend on the balance of carbon (C) acquisition, nutrient availability, temperature, soil moisture and other factors. Globally, terrestrial ecosystems presently take up about one quarter of the anthropogenic CO₂ emissions (Le Quéré et al. 2013), thereby dampening the increase in atmospheric CO₂ and limiting the increase in global temperature (Wang and Houlton

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2009). Many terrestrial ecosystems are limited in their productivity by the availability of nitrogen (N) for plant growth (LeBauer and Treseder 2008). Therefore, the future response of these ecosystems to global changes as well as their ability to take up and store C may be limited by N availability (Hungate et al. 2003). Indeed, several experiments have demonstrated that N-limitation can prevent any stimulation of productivity by elevated CO₂ (Norby et al. 2010; Reich and Hobbie 2013). Further, if increased CO₂ concentration does lead to a stimulation of biomass production, then this could cause a shift in the distribution of N in the terrestrial ecosystem towards long-lived biomass and litter pools, reducing N availability and as a consequence biomass stimulation. This has been formalized in the hypothesis of a progressive N limitation (PNL) of ecosystems under elevated CO₂ (Luo et al. 2004). However, ecosystems may respond to global change by feedbacks that increase N availability, hence maintaining C uptake as CO₂ concentrations increase. Such mechanisms include increased N₂ fixation (Hu et al. 2006), mining for mineral N in deeper soil layers (Iversen et al. 2011) and increasing mineralization-immobilization turnover (Rütting and Andresen 2015; Rütting 2017). Moreover, responses of the N cycle in terrestrial ecosystems to climate change might differ depending on whether the ecosystem is limited by N or phosphorus (P) (Dijkstra et al. 2013; Rütting and Andresen 2015).

Awareness has been raised of the interactive effects of elevated CO₂ and warming on terrestrial ecosystem processes (Leuzinger et al. 2011; Dieleman et al. 2012), including soil N cycle processes (Larsen et al. 2011). These studies demonstrated that simple additive effects of multiple climate change factors are rare, but that antagonistic or synergistic effects are many. This hinders our predictions of ecosystem responses to climate change factors based on single treatment experiments. In particular, few studies have examined the combined effects of warming and elevated CO₂ on the soil N cycle (Hovenden et al. 2008; Larsen et al. 2011; Niboyet et al. 2011; Björnsne et al. 2014).

The plant community might also affect how ecosystem processes respond to climate change. Grasslands are often complex communities of different groups of plant functional types, including C3 and C4 grasses, legumes and other forbs. Soussana and Lüscher (2007) hypothesized that C3 plants would be favoured by elevated CO₂, as their photosynthesis is

not yet CO₂ saturated. On the other hand, C4 plants are expected to be favoured by warming since this plant group has evolved mechanisms to save water under warm conditions. However, in the long-term these expected responses based on plant physiology could be overwhelmed by biogeochemical responses (Reich et al. 2018). These potentially different responses of the vegetation can also be important for the alteration of belowground processes (Pendall et al. 2011).

For a Tasmanian grassland (TasFACE experiment; FACE = free air CO₂ enrichment) it was shown that N availability, measured by ion exchange membranes, was generally decreased under elevated CO₂ (Hovenden et al. 2008, 2017). Moreover, an interaction with warming was found; N availability increased by warming under elevated CO₂, but not under ambient CO₂. However, in the long-run of that experiment warming acted independently of CO₂ level, leading to doubled N availability in warmed plots compared to unwarmed plots (Hovenden et al. 2017). The changes in N availability were independent of plant growth responses, as generally plant growth was unaffected by climate change treatments (Hovenden et al. 2017). It was also found that vegetation type (C3 vs. C4 grasses) affected the responses of decomposition and nutrient release in the soil to simulated climate change (Osanaï et al. 2015).

In the absence of changed plant N uptake, changes in plant N availability can originate either from altered production of plant available N (i.e. N mineralization) or consumption (i.e. microbial immobilization). The mechanism can be revealed by investigating gross soil N transformations. Neither the TasFACE nor, to our knowledge, any other climate change experiment have reported the combined effects of elevated CO₂, warming and vegetation type on gross soil N dynamics, which is the aim of the present study. Our hypotheses were that gross N mineralization would decrease by elevated CO₂ and would increase by warming, resulting in changed N availability (Hovenden et al. 2017), while gross nitrification is rather unresponsive to climate change. We further hypothesize that the responses to combined climate treatments are interactive and, more specific, antagonistic, based on findings in other experiments (e.g. Larsen et al. 2011).

Material and methods

Site description

The present study was conducted using the TasFACE climate change experiment, in which a native Tasmanian grassland is exposed to elevated CO_2 and to elevated air temperature in a two-factor design (Hovenden et al. 2006). The TasFACE site has a Mediterranean climate with annual mean temperature of 11.6 °C and a mean annual precipitation of ~ 400 mm with a significant summer drought. The soil at the site is a black Vertosol (Isbell 2002). The climate change treatments are achieved by mini-FACE technology (1.5 m ring diameter), exposing the ecosystem to either ambient or elevated $[\text{CO}_2]$ of 550 ppm, and by infrared radiation using infrared emitters installed 1.2 m above soil surface, resulting in an increased soil temperature at 1 cm depth of 0.82 °C over the growing season (Hovenden et al. 2008). Each treatment was replicated three times, resulting in a total of twelve treatment plots. The climate manipulations started in February 2002 and soil sampling was conducted from all replicate plots in November 2011, at the termination of the experiment to investigate the accumulated effects of 10 years of climate manipulation on the gross soil N dynamics. The grassland vegetation of the site is species rich with 51 vascular species recorded and the dominant perennial grasses are the C4 grass *Themeda triandra* (the only recorded C4 grass) and the C3 grasses *Rytidosperma caespitosa* and *R. carphoides* (Hovenden et al. 2006). Soil samples were collected separately from under the C4 grass (*Themeda*) and under C3 grasses (non-*Themeda*) in each plot (Pendall et al. 2011). Three to four soil samples per ring and vegetation type were taken with a 35 mm auger to a depth of 5 cm and then bulked. The soil depth was chosen because more than 80% of all roots occur in that soil layer. Soil samples were stored cool during shipping and stored until the start of the tracer experiment.

^{15}N tracer experiment

About 1 month after soil sampling, all samples ($N = 24$) were sieved with a mesh size of 2 mm, to remove larger roots and other visual debris. An equivalent of 20 g dry soil was then weighed into 200 mL glass bottles, which were covered by parafilm,

containing five small holes to allow gas exchange, and were pre-incubated in a climate chamber (Percival, CLF PlantClimatics) at 15 °C in darkness for three days prior to the ^{15}N tracer addition. The temperature was kept constant throughout the whole incubation. For the tracer addition, all soil samples received an amount of 0.5 $\mu\text{g NO}_3^- - \text{N}$ and 1 $\mu\text{g NH}_4^+ - \text{N}$ per gram soil dry weight, which was applied dissolved in 3 mL of distilled water. Half of the samples received ^{15}N tracer in the form of $^{15}\text{NH}_4^+$ and the other half in the form of $^{15}\text{NO}_3^-$, ^{15}N enriched at 99%.

Soil samples were extracted at 0.25 and 20 h after tracer addition, by adding 2 M KCl in a ratio of 1:2 (soil to liquid). The soil/KCl slurries were shaken for 1 h on a vertical shaker and subsequently filtered through Whatman GF/D glass fibre filter paper. The mineral N content and their respective ^{15}N enrichment in the filtrates were analysed using the SPINMAS technique (Stange et al. 2007). The SPINMAS combines an automatized sample preparation unit (SPIN), in which inorganic N is chemically transformed to a gaseous N species, with a quadrupole mass spectrometer (GAM400, InProcess, Bremen). Prior to soil extraction, a subsample was taken to determine the soil moisture, the soil organic matter content (SOM) by loss on ignition at 550 °C, total C and N content (TC and TN) using an elemental analyser (EA 1108, Fison Instrument, Italy) and total P using an ICP-ES (at ACME Labs, Vancouver, Canada). Soil pH was measured in 2 M KCl extracts (1:4 soil to liquid).

Statistical considerations

Quantification of gross N mineralization and gross nitrification was conducted using the analytical tracing model by Kirkham and Bartholomew (1954), using the data from the $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ labelling treatment, respectively. We observed occasionally negative gross rates for nitrification, which are biological impossible. There is inconsistency in the literature how to treat those, but we decided to set all negative rates to zero. However, average gross nitrification including the negative rates are presented in Supplementary Fig. 1.

Gross N transformations and physico-chemical soil properties were analysed for significant differences via multi-way nested Analysis of Variance (ANOVA), with CO_2 level and temperature as main variables and vegetation type nested within CO_2 and temperature. Data were checked for normality and equal variance

Table 1 Physico-chemical soil properties (mean $\pm$ standard deviation; N = 3) from the TasFACE climate change experiment (0–5 cm depth)

Treatment ^a	GWC (%)	pH	SOM (%)	C (%)	N (%)	C/N	P (%)
aCaT-C3	19.9 $\pm$ 4.0	6.0 $\pm$ 0.1	8.1 $\pm$ 1.3	2.8 $\pm$ 0.6	0.19 $\pm$ 0.05	14.6 $\pm$ 0.3	0.016 $\pm$ 0.002
aCaT-C4	19.4 $\pm$ 0.8	6.1 $\pm$ 0.1	8.4 $\pm$ 0.6	3.2 $\pm$ 0.4	0.22 $\pm$ 0.04	14.6 $\pm$ 0.8	0.017 $\pm$ 0.001
aCeT-C3	16.7 $\pm$ 5.1	6.1 $\pm$ 0.1	7.5 $\pm$ 1.6	2.7 $\pm$ 0.6	0.19 $\pm$ 0.04	14.2 $\pm$ 0.9	0.014 $\pm$ 0.003
aCeT-C4	20.0 $\pm$ 4.6	6.1 $\pm$ 0.1	9.2 $\pm$ 2.5	3.6 $\pm$ 1.2	0.25 $\pm$ 0.08	14.7 $\pm$ 0.3	0.018 $\pm$ 0.003
eCaT-C3	19.3 $\pm$ 2.6	5.8 $\pm$ 0.2	7.5 $\pm$ 0.6	3.1 $\pm$ 0.1	0.19 $\pm$ 0.00	15.9 $\pm$ 0.5	0.015 $\pm$ 0.002
eCaT-C4	20.7 $\pm$ 2.6	5.8 $\pm$ 0.1	7.8 $\pm$ 1.4	3.1 $\pm$ 0.6	0.20 $\pm$ 0.04	15.8 $\pm$ 0.4	0.015 $\pm$ 0.003
eCeT-C3	16.5 $\pm$ 2.4	5.8 $\pm$ 0.1	7.7 $\pm$ 0.2	3.3 $\pm$ 0.6	0.22 $\pm$ 0.04	15.1 $\pm$ 0.5	0.015 $\pm$ 0.001
eCeT-C4	19.5 $\pm$ 2.9	5.9 $\pm$ 0.2	8.4 $\pm$ 0.7	3.3 $\pm$ 0.4	0.22 $\pm$ 0.03	15.1 $\pm$ 0.5	0.016 $\pm$ 0.002

There were no significant effects of carbon dioxide (CO₂) level, temperature or vegetation, with the exception of a significant effect ($P < 0.05$) of CO₂ on carbon-to-nitrogen (C/N) ratio and soil pH and a tendency for a warming effect ($P < 0.1$) on C/N ratio (see Supplementary Table 1 for details on statistics)

^aTreatments are: *aCaT* ambient [CO₂] and temperature, *aCeT* ambient [CO₂] and elevated temperature, *eCaT* elevated [CO₂] and ambient temperature, *eCeT* elevated [CO₂] and temperature; C3 and C4 denote vegetation type

prior to statistical analysis. Pearson correlation and linear regression was applied to identify soil properties that can explain variation in gross N transformations. Statistical analysis were conducted using Matlab R2013b (The MathWorks, Inc.; ANOVA) or SigmaPlot (Version 11, Systat Software, Inc.).

To investigate if a common interactive effect of combined elevated CO₂ and warming on gross N cycling exists, we compiled the available data from grassland FACE experiments with factorial warming. Those data came from three climate change experiments (Table 2), with a total of 9 data points for gross N mineralization and 7 data points for gross nitrification. We calculated the percentage change of a climate treatment (elevated CO₂, warming and elevated CO₂ $\times$ warming) compared to ambient condition. To investigate if, as hypothesized, antagonistic effects are common we plotted the change in gross N rates under combined elevated CO₂ and warming against the sum of changes for the single factor treatments. If the effects of climatic treatments are additive, the points should follow the 1:1 line. We further conducted a bivariate line fitting (Warton et al. 2006) using the standardized major axis in the SMATR software (Falster et al. 2003) and we tested if the slope significantly deviated from one, which would indicate antagonistic (slope < 1) or synergistic (slope > 1) effects. This was done separately for gross N mineralization and nitrification.

Results and discussions

Physico-chemical soil properties were in general unaffected by climate change treatments and were not different between vegetation types (Table 1; Supplementary Table 1). The only significant effects were a higher C/N ratio ($F = 17.56$, $P < 0.001$) and a lower soil pH ($F = 23.07$, $P < 0.001$) under elevated CO₂, while warming tended to decrease the soil C/N ratio ($F = 3.15$, $P = 0.095$).

In the present study, average gross N mineralization ranged from 4.9 to 11.3 $\mu\text{g N g}^{-1} \text{ day}^{-1}$ (Fig. 1) and NH₄⁺ consumption from 6.3 to 12.2 $\mu\text{g N g}^{-1} \text{ day}^{-1}$. Gross nitrification was about 10-times lower (0.22 to 0.97 $\mu\text{g N g}^{-1} \text{ day}^{-1}$; Fig. 1), indicating a closed N cycle (Aber 1992). Across treatment, the best predictors for average gross N mineralization were the two soil factors that were significantly affected by CO₂ level, pH ($R^2 = 0.39$) and C/N ratio ($R^2 = 0.33$), where pH was positively and C/N ratio negatively correlated with gross N mineralization. None of the measured physico-chemical soil properties (see Table 1) was strongly correlated ($r > 0.5$) to gross nitrification.

In contrast to our hypothesis, we did not find any significant effect of climatic treatments or vegetation type on gross N transformations (Supplementary Table 2). This is in line with the generally small changes in plant growth in the TasFACE experiment (Hovenden et al. 2014), which did consequently not

Fig. 1 Average (+ standard deviation) gross nitrogen mineralization (upper) and gross nitrification (lower) rates in a native Tasmanian grassland subjected to climate change treatments warming by infrared heaters or elevated carbon dioxide (CO₂)

induce a “priming” of gross N mineralization. Previous studies reported variable responses of gross soil N transformations to elevated CO₂ (De Graaff et al. 2006; Rütting and Andresen 2015) and warming. Similar to our results, gross N mineralization was unaffected by warming in a Dutch heathland (Andresen et al. 2015). On the other hand, both increased (Larsen et al. 2011; Björnsne et al. 2014) and decreased

by Free Air CO₂ enrichment (single or in combination). Gross rates were separately investigated in soil collected under C3 and C4 vegetation (N = 3). Negative gross rates for nitrification were set to zero prior to averaging

(Jamieson et al. 1998; Niboyet et al. 2011) gross N mineralization under warming has been reported. Variable N cycling responses to warming have even been detected within experimental sites related to season or duration of warming (Shaw and Harte 2001; Jamieson et al. 1998). However, in a Danish heathland a similar response of gross N mineralization to both warming and elevated CO₂ was observed after 2, 5 and

Table 2 Overview over Free Air CO₂ Enrichment (FACE) experiments that have investigated gross nitrogen dynamics responses to combined elevated CO₂ and warming

Experiment	Code	Exp. year	[CO ₂] _e (ppm)	Depth (cm)	Add. treatment	Method details ^a	References
CLIMATE	C_Y2	2	510	0–5		IPD; lab; 7 days; 20 °C	Larsen et al. (2011)
	C_Y5	5	510	0–8		Trace; lab; 7 days; 22 °C	Björnsne et al. (2014)
	C_Y8	8	510	0–5		IPD; lab; 24 h; 10 °C	Wild et al. (2018)
JRGCE	J_F–	7	780	0–5	Low-N Feb	IPD; field; 24 h	Niboyet et al. (2011)
	J_F +	7	780	0–5	High-N Feb		
	J_A–	7	780	0–5	Low-N Apr		
	J_A +	7	780	0–5	High-N Apr		
TasFACE	T_C3	7	550	0–5	C3 vegetation	IPD; lab; 24 h; 15 °C	This study
	T_C4	7	550	0–5	C4 vegetation		

^a¹⁵N labelling (IPD = isotope pool dilution; Trace = ¹⁵N tracing); conditions (laboratory or field); length of incubation; Incubation temperature

8 years of manipulation (Larsen et al. 2011; Björnsne et al. 2014; Wild et al. 2018). Reasons for these variable responses to climate change might be changes or the absence of changes in C and N inputs to soil or changes in plant litter depending on the plant responses to climate change (Jamieson et al. 1998). For the TasFACE sites only small changes in plant biomass with climate manipulations have been observed (Hovenden et al. 2014), which might explain the unresponsiveness of the gross soil N cycle.

One of our aims was to investigate if interactive effects between warming and elevated CO₂ occurred. In contrast to our hypotheses, we observed no interactive effects of elevated CO₂ and warming for gross N mineralization or nitrification (Supplementary Table 2), possibly due to the overall unresponsiveness of gross rates to the climate change treatments. Previously, an interactive effect was shown for C mineralization capacity at the TasFACE, which was only affected by combined warming and elevated CO₂, but not by the factors in isolation (Osanai et al. 2015). These observations point to a potential decoupling of C and N cycling responses to climate change at the TasFACE site. Possible explanations for the contrasting effects of climate change factors on the C and N cycling are changes in plant C/N ratios (Pendall et al. 2011) or in the microbial community (Osanai et al. 2015). Elevated CO₂ often leads to enhanced belowground C allocation (e.g. Reinsch et al. 2014; Pendall et al. 2004), possibly also in the case when plant growth is not stimulated. Such an increased

rhizodeposition of C can alleviate microbial C limitation in soil. As gross N mineralization is the release of excess N in relation to C during the decomposition of SOM, enhanced rhizodeposition could decrease gross N mineralization. The observed increase in soil C/N ratio under elevated CO₂ (Table 1) might explain the tendency of gross N mineralization to decrease under elevated CO₂ at the TasFACE.

To explore how the observed pattern for gross N rates in this study are in agreement with other grassland experiments with combined FACE and warming (Table 2), we compared the change in gross rates under combined elevated CO₂ and warming with the sum of the responses to single treatments (Fig. 2). Additive effects were extremely rare and antagonistic effects were the dominant response for both, gross N mineralization and nitrification, as most points lie between the 1:1 line and the x-axis (Fig. 2). Antagonistic effects on gross N transformation have also been observed in a rice paddy study (Chen et al. 2016). The slopes of the bivariate line fitting for gross N mineralization (0.525) and gross nitrification (0.351) were both significantly different from one ($P = 0.034$ and 0.013 , respectively), further supporting the existence of antagonistic responses. Antagonistic responses of combined elevated CO₂ and warming have been identified as common for different ecosystem processes, while additive effects are generally rare (Larsen et al. 2011; Leuzinger et al. 2011; Dieleman et al. 2012). Here we show that this is also the case for gross N mineralization and nitrification in grassland

Fig. 2 Results from bivariate line fitting (solid line) between the response (percentage change) of combined elevated CO₂ and warming and the sum of response to single treatments for gross N mineralization (upper panel) and gross nitrification (lower

panel). In both cases, the slope significantly differed from 1. Dashed line indicates the 1:1 line. For codes of experiments, see Table 2

soils. Consequently, the relative change of gross N cycling rates is lower than what would be predicted from single treatment experiments. As relatively few experiments so far have combined elevated CO₂ and warming on an ecosystem scale there is an urgent need for more such experiments, to establish a more robust

and realistic understanding on climate change effects on terrestrial ecosystem processes.

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