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Regulation of CO_2 and N_2O fluxes by coupled carbon and nitrogen availability

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Abstract

Carbon (C) and nitrogen (N) interactions contribute to uncertainty in current biogeochemical models that aim to estimate greenhouse gas (GHG, including CO_2 and N_2O) emissions from soil to atmosphere. In this study, we quantified CO_2 and N_2O flux patterns and their relationship along with increasing C additions only, N additions only, a C gradient combined with excess N, and an N gradient with excess C via laboratory incubations. Conventional trends, where labile C or N addition results in higher CO_2 or N_2O fluxes, were observed. However, at low levels of C availability, saturating N amendments reduced soil CO_2 flux while with high C availability N amendments enhanced it. At saturating C conditions increasing N amendments first reduced and then increased CO_2 fluxes. Similarly, N_2O fluxes were initially reduced by adding labile C under N limited conditions, but additional C enhanced N_2O fluxes by more than two orders of magnitude in the saturating N environment. Changes in C or N use efficiency could explain the altered gas flux patterns and imply a critical level in the interactions between N and C availability that regulate soil trace gas emissions and biogeochemical cycling. Compared to either N or C amendment alone, the interaction of N and C caused ~60 and ~5 times the total GHG emission, respectively. Our findings suggested that the response of CO_2 and N_2O fluxes along stoichiometric gradients in C and N availability should be accounted for interpreting or modeling the biogeochemistry of GHG emissions.

1. Introduction

Carbon dioxide (CO_2) and nitrous oxide (N_2O) are major greenhouse gases (GHGs) that produce a strong positive radiative forcing in the atmosphere. Extensive work has been directed to understanding single substrate dependences of CO_2 on organic carbon (C) and N_2O to nitrogen (N), both experimentally and through modeling of microbial CO_2 and of N_2O emissions (Davidson *et al* 2012, Manzoni *et al* 2012, Liu *et al* 2012, Jassal *et al* 2005, Signor *et al* 2013, Burzaco *et al* 2013). However, the plasticity of C and N metabolism in microorganisms (Ter Schure 2000, Horák 1997) produces large uncertainties in coupling of either CO_2 or N_2O trace gas emissions to single substrate availability.

Recent evidence suggests N availability can influence CO_2 production and in turn C availability may influence N_2O emissions (Piao *et al* 2013, Jain

et al 2013, Liu and Greaver 2009). Studies have generally not evaluated emissions of both trace gases simultaneously, although potential interactions between substrate availabilities may lead to important connections between the two fluxes through a coupling of the C and N biogeochemical cycles (Sokolov *et al* 2008, Thornton *et al* 2009, Bonan and Levis 2010, Zaehle and Dalmonech 2011, Lal 2008). The interactive influence of C and N substrate dependences on the biogeochemical processes mediating soil CO_2 and N_2O fluxes remains a key uncertainty in understanding the regulation and magnitude of GHG emissions from soils.

As directly measured byproducts of microbial C or N metabolism, CO_2 and N_2O fluxes provide a window to inspect the energy (C) and nutrient (e.g. N) allocation of soil microorganisms through direct relationships with C and N use efficiency (CUE and NUE). In ecological stoichiometry, CUE or NUE is commonly applied to quantify the balance of C or N between

biomass growth and consumption (Mooshammer *et al* 2014, Manzoni *et al* 2012, Roland and Cole 1999). In general, a high CUE or NUE means an increasing microbial biomass but slowed C or N mineralization rate, resulting in low soil CO_2 or N_2O fluxes. In contrast, a low CUE or NUE indicates an inefficient conversion of C or N to biomass, a large return of C or N to the environment, and increased soil CO_2 or N_2O fluxes. A limiting C substrate produces a relatively high CUE while a limiting N source can reduce the CUE, a consequence of coupling or uncoupling of microbial catabolism and anabolism (Sinsabaugh *et al* 2013). Microbial NUE is likely controlled and regulated similarly to CUE but directly coupled to the N cycle and associated emissions of N trace gases (Mooshammer *et al* 2014). Thus, variation in CO_2 or N_2O flux patterns can be used as an assessment of CUE or NUE under different C or N levels (Eberwein *et al* revised). Because of the intrinsic linkage between microbial C and N metabolism (Richardson 2000, Robertson and Groffman 2007), how the overlap between microbial CUE and NUE simultaneously mediates CO_2 and N_2O fluxes needs evaluation.

In this study, we conducted a series of soil incubations to identify the potential interactions between soil CO_2 and N_2O emissions in response to variation in labile C and N amendments. We asked: 1) are soil CO_2 and N_2O soil emissions dependent on the availability of both C and N, and 2) are emissions of the two trace gasses correlated in their flux rates? Answering these questions will test alternate hypotheses of trace gas emission regulation, 1) a single-substrate hypothesis currently used in most trace gas emissions models that predicts regulation by a single resource and 2) a dynamic efficiency hypothesis for C and N that predicts interactions between resources will regulate both CO_2 and N_2O fluxes. The results from this study will improve understanding of how both C and N biogeochemical cycles are influenced by multiple limiting resources and demonstrate the potential coupling between these biogeochemical cycles with direct consequences for total GHG emissions.

2. Materials and methods

2.1. Soil characterization
The soil used for our study was collected from an agricultural field (13 ha) located at the University of California Desert Research and Extension Center, El Centro, California (32°N 48′ 42.6′, 115°W 26′ 37.5″). The site is a high temperature, low elevation, desert environment with mean annual precipitation of 5.8 mm and monthly mean air temperatures between 13.9 and 33.9 °C, and extremely high midday temperatures up to 50 °C (www.weather.com). The site has deep alluvial soils (42% clay, 41% silt, 16% sand) with 2.34% C and 0.13% N, and a *p*H of 8.3 (Oikawa *et al* 2014). Prior to soil collection, the field was fallow for 8 months then planted with forage sorghum for

two years. Soils were collected between 0–10 cm depths from 5 random locations in the field.

2.2. Laboratory incubations
Prior to incubations, the soil was air dried in the lab, sieved (2 mm mesh), and then homogenized. Soil water holding capacity (WHC) was determined by the gravimetric method (Pansu and Gautheyrou 2006). Three replicate samples (100 g dry weight) were placed in glass jars (~473 ml) and maintained a 40% WHC by weighing the jar every two days and adding de-ionized water as necessary during the incubation period.

To investigate soil CO_2 and N_2O flux responses to C and N amendments and their interaction, two series of laboratory incubations were conducted that included a control (de-ionized water only), dextrose (as a labile carbon source) only, N (ammonium nitrate, NH_4NO_3) only, and both dextrose and N. The first series of incubations (Experiment 1, Exp1) were conducted to quantify soil CO_2 and N_2O fluxes under six levels of N amendment with two levels of C amendment (with and without C). Six levels of N amendment as 0, 10, 50, 200, 700, or 1500 $\mu g \, N \, g^{-1}$ soil were selected. Along the N gradient, a control and saturating C level (60 g L^{-1} dextrose, which is equivalent to 18 mg g^{-1} soil) were selected to investigate C and N interactions. Each treatment included three replicates with 36 samples in total. For the second series of incubations (Experiment 2, Exp2), another 36 samples were used to investigate CO_2 and N_2O fluxes under different C level with saturating N supplement. A C amendment of 0, 1.5, 3, 7, 18, or 30 mg dextrose g^{-1} soil was set and combined with either no N or a saturating N level (700 $\mu g \, N \, g^{-1}$ soil). All 72 jars were incubated at 25 °C in the lab for 7 days and CO_2 and N_2O fluxes were measured daily.

2.3. Flux measurements
We used a flux measurement system that allowed simultaneous measurements of both CO_2 and N_2O trace gases within a total sampling period of less than five minutes. Soil N_2O emissions have typically relied on syringe extraction over a thirty minute to one hour sampling period at minimum and subsequent analysis on a gas chromatograph (Alves *et al* 2012, Dobbie and Smith 2003). Our system provides the capability to measure the instantaneous fluxes of N_2O and CO_2 and allows investigation of the potential relationships between CO_2 and N_2O fluxes.

We built a dynamic closed system (figure 1) to measure CO_2 and N_2O fluxes simultaneously with a Li-7000 infrared gas analyzer for CO_2 (Licor Biosciences, Lincoln, Nebraska, USA) connected to a N_2O gas analyzer (913-EP, Los Gatos Research, Mountain View, California, USA). The N_2O analyzer uses off-axis integrated cavity output spectroscopy (Off-Axis ICOS) to provide a real-time accurate N_2O concentration measurement with a precision of 0.05 ppb at 1 hz sampling frequency. The CO_2 and N_2O fluxes were
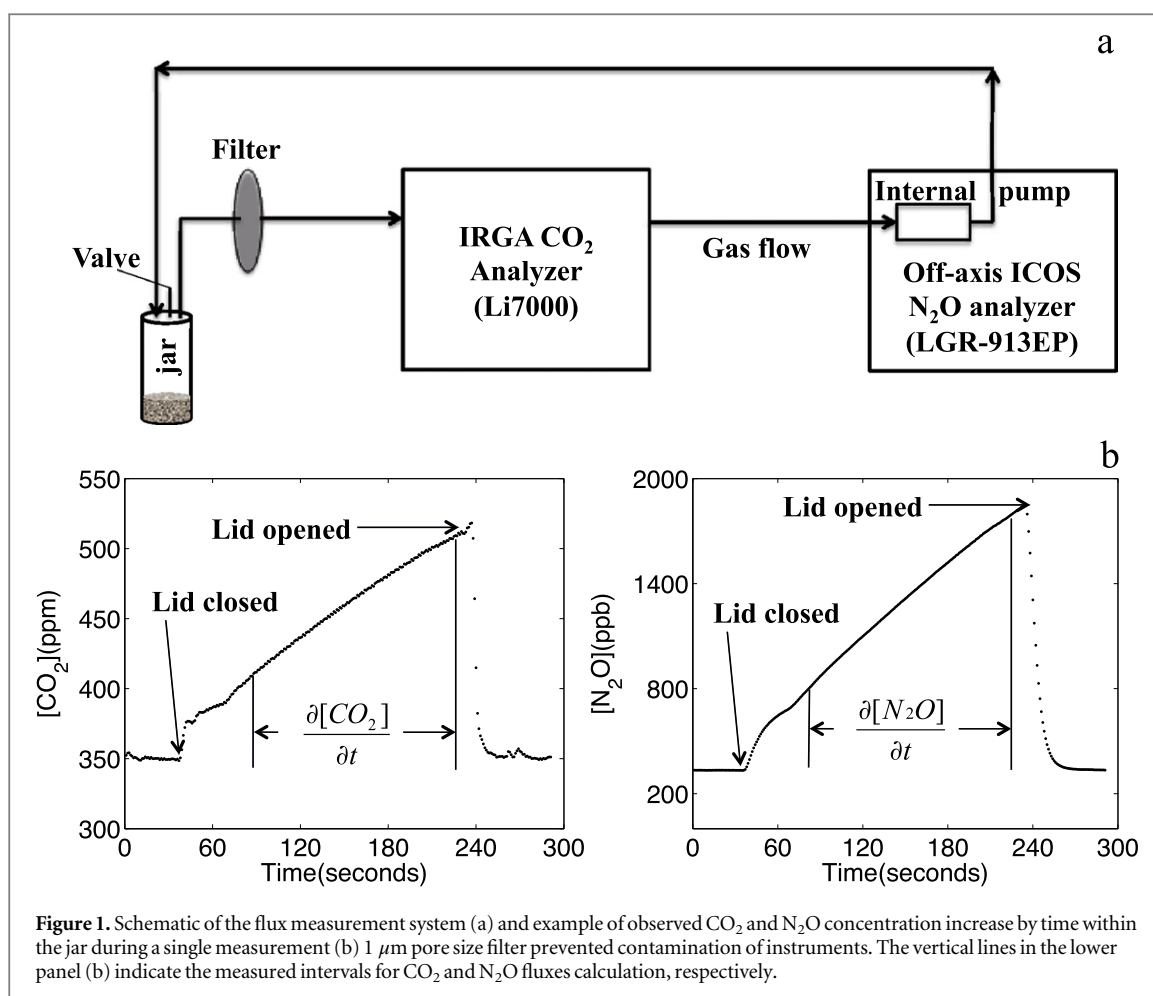
Figure 1. Schematic of the flux measurement system (a) and example of observed CO_2 and N_2O concentration increase by time within the jar during a single measurement (b) 1 μm pore size filter prevented contamination of instruments. The vertical lines in the lower panel (b) indicate the measured intervals for CO_2 and N_2O fluxes calculation, respectively.

determined by the linear regression fit between the CO_2 or N_2O concentration change and the measured time. For CO_2 flux calculation, the original model from Licor (Licor 8100 Manual) was adapted for our jar measurements as follows:

$$F_c = \frac{VP_0\left(1 - \frac{w_0}{1000}\right)}{RM_s\left(T_0 + 273.15\right)} \frac{\partial\left[CO_2\right]}{\partial t}, \qquad (1)$$

where F_c is CO_2 flux (μmol CO_2 g^{-1} soil s^{-1}) from the soil in the jar. V (cm^3) is the volume difference between the jar plus the tubing and the soil (calculated using a bulk density value 1.15 g cm^{-3} of our soil). P_0 is the initial pressure (kPa). w_0 is initial water vapor in mole fraction (mmol mol^{-1}). R is the ideal gas constant (8.314×10^3 kPa cm^3 K^{-1} mol^{-1}). M_s is the mass of soil (g) and T_0 is the initial air temperature (°C). The factor $\frac{\partial\left[CO_2\right]}{\partial t}$ is the changing rate of CO_2 concentration along time (μmol mol^{-1} s^{-1}). The N_2O flux was calculated using the same method but the dry N_2O concentration reported from N_2O analyzer during the measured intervals was used and thus the water correction term in equation (1) was not needed. Both trace gas measurements were completed within 3–10 mins depending on flux rate. Seven day cumulative CO_2 and N_2O fluxes were calculated by interpolating the measurements from each day and then integrating.

2.4. Statistics

We performed two-way fixed-model ANOVA to test the response of the seven day cumulative CO_2 and N_2O fluxes to carbon and nitrogen addition. Prior to conducting ANOVA, the normality of the data and the homogeneity of variances were tested using the Shapiro–Wilk test (Royston 1982, Shapiro and Wilk 1965) and the Levene's test (Brown and Forsythe 1974, Levene 1960), respectively. The Tukey's honestly significant difference (HSD) test (Tukey 1949) was used to examine intra-group differences. When necessary, Box–Cox transformations (Box and Cox 1964) were applied to meet the assumptions of ANOVA. For N_2O fluxes, we added a constant positive value to meet the logarithmic transformation because of some negative values observed during the incubation in association with low rates of net uptake (Majumdar 2013). All statistical analyses and data processing were performed using MATLAB R2011b (The MathWorks Inc., Natick, MA, USA) and the R package (R Core Team 2013).

3. Results

3.1. CO_2 flux

Cumulative CO_2 flux was significantly affected by C ($p < 0.0001$ in Exp1 and Exp2) and N ($p < 0.0001$

Figure 2. Soil CO_2 flux response to dextrose and nitrogen gradient. Panel a illustrates the CO_2 flux response to increasing C amendments with and without saturating N additions (at 700 $\mu g\,g^{-1}$ soil). Panel c shows CO_2 response to increasing N amendments with and without saturating dextrose (at 18 mg g^{-1} soil). Panel b shows the CO_2 flux difference with N amendment (with N minus no N) at increasing C levels and d shows the difference with C amendment (with dextrose minus no dextrose) at increasing N levels. The significance level is set to $p = 0.05$.
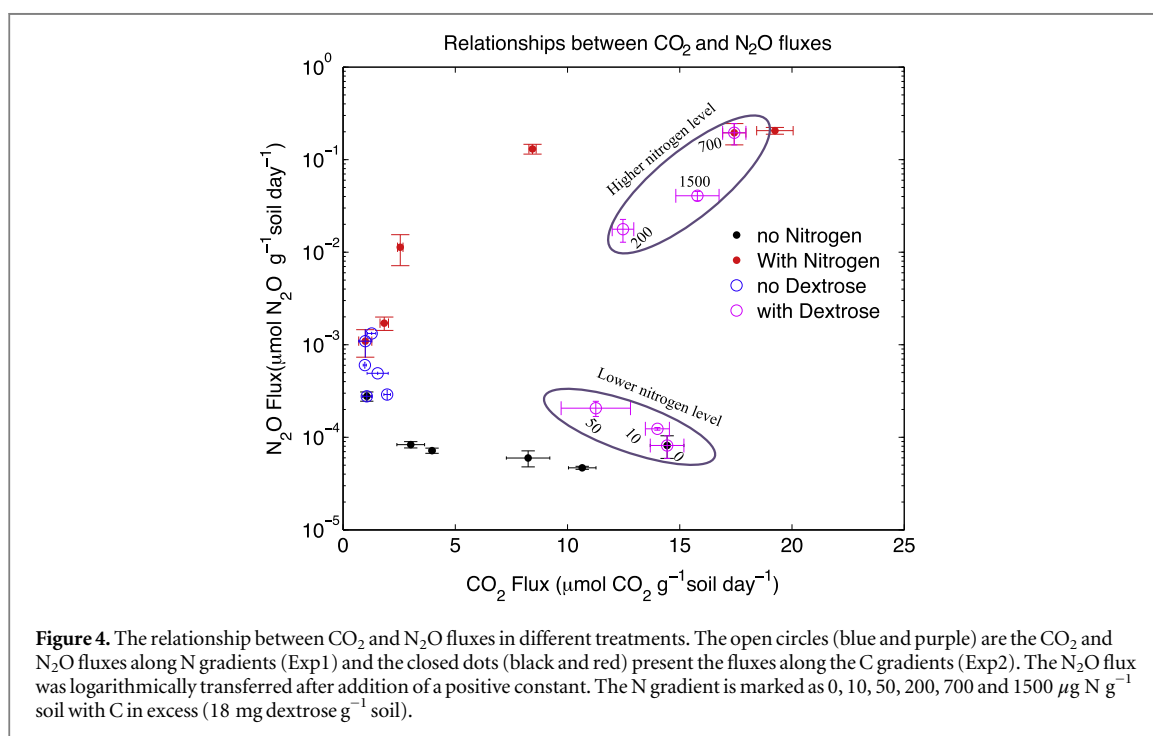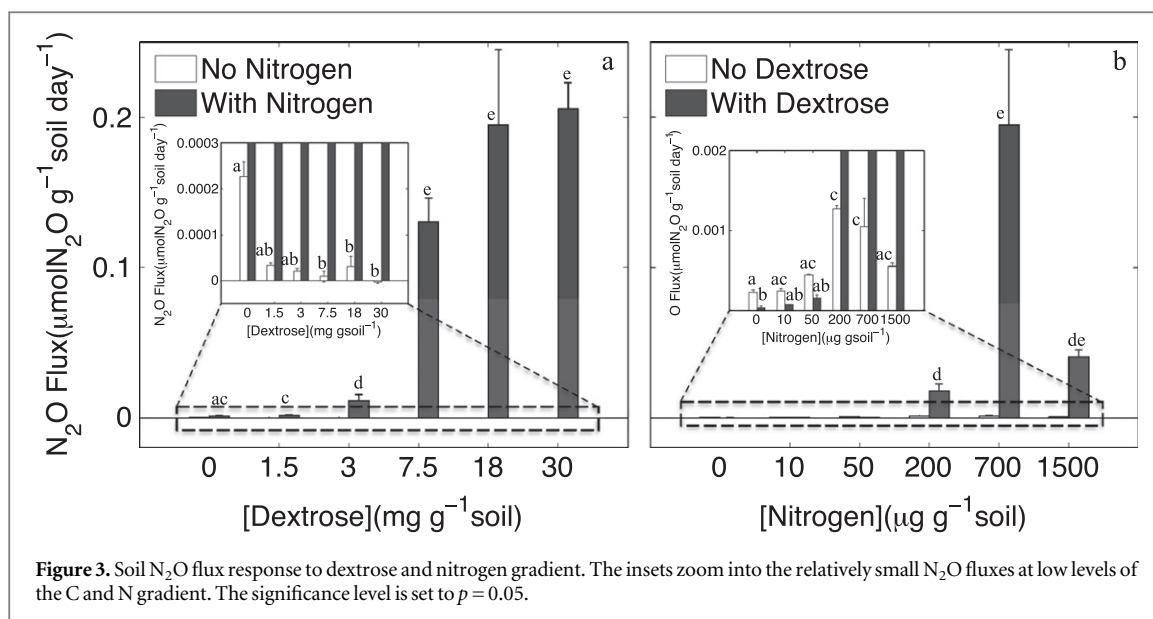
in Exp1 and $p = 0.0006$ in Exp2) amendments and their interaction effects ($p < 0.0001$ in Exp1 and Exp2) based on a two-way ANOVA. C effects on CO_2 flux were positive, indicating a higher dextrose concentration produced higher CO_2 flux (figure 2(a)). However, the N effects on CO_2 flux were diverse. Under saturating N conditions, the CO_2 flux was reduced 39% and 36% at low dextrose levels (1.5 and 3 mg g^{-1} soil, respectively) compared to treatments without any N amendment (figures 2(a)–(b) without N). But high dextrose levels (18 and 30 mg g^{-1} soil) resulted in significantly higher CO_2 fluxes with N addition compared to without N (figures 2(a)–(b) with N). Although no significant decrease in CO_2 flux was found at lower dextrose levels (1.5 and 3 mg g^{-1} soil) with N addition, there was a significant decrease in CO_2 flux at lower N addition when dextrose was saturating (figures 2(c)–(d) with dextrose). CO_2 flux increased at a high but sub-toxic N level (700 $\mu g\,g^{-1}$ soil) and decreased at an inhibitory N level (1500 $\mu g\,g^{-1}$ soil). However, there were no significant effects of N addition on CO_2 flux when dextrose was not added (figure 2(c) no dextrose).

3.2. N$_2$O flux

Cumulative N_2O fluxes were significantly affected by amendments of C ($p < 0.0001$) and N ($p < 0.0001$) and their interaction ($p < 0.0001$) in both experiments. N amendments had consistent positive effects on N_2O fluxes (figures 3(a), (b). However, additions of C led to contrasting N_2O flux responses. When N additions were low (control, 10 and 50 $\mu gN\,g^{-1}$ soil), the additional C source significantly reduced N_2O fluxes (figure 3(a) no nitrogen and figure 3(b) with dextrose) but increased fluxes under a higher N level (200 and 700 $\mu gN\,g^{-1}$ soil) (figure 3(a) with nitrogen and figure 3(b) with dextrose) until N additions became inhibitory (1500 $\mu gN\,g^{-1}$ soil). Thus, N_2O fluxes were reduced by adding extra dextrose under a limited N condition but were dramatically increased when both N and C resources were sufficient.

3.3. Relationships between CO$_2$ and N$_2$O fluxes

Comparing CO_2 and N_2O fluxes under different conditions, there was a clear opposite relationship between cumulative CO_2 and N_2O fluxes demonstrated in this study (figure 4). When N was not added,

Figure 3. Soil N_2O flux response to dextrose and nitrogen gradient. The insets zoom into the relatively small N_2O fluxes at low levels of the C and N gradient. The significance level is set to $p = 0.05$.

Figure 4. The relationship between CO_2 and N_2O fluxes in different treatments. The open circles (blue and purple) are the CO_2 and N_2O fluxes along N gradients (Exp1) and the closed dots (black and red) present the fluxes along the C gradients (Exp2). The N_2O flux was logarithmically transferred after addition of a positive constant. The N gradient is marked as 0, 10, 50, 200, 700 and 1500 μg N g^{-1} soil with C in excess (18 mg dextrose g^{-1} soil).

additional C increased CO_2 fluxes (figure 2(a) no nitrogen) but reduced N_2O fluxes (figure 3(a) no nitrogen), resulting in a negative relationship between them. In contrast, when N availability was saturating, CO_2 and N_2O fluxes increased with additional C amendments from low to high (figures 2(a) and 2(a) with nitrogen), resulting in a positive relationship. In an unlimited C environment, a negative N effect on CO_2 flux and a positive N effect on N_2O flux resulted in a positive relationship between CO_2 and N_2O fluxes. When N crossed a critical level (between 50–200 $\mu g N$ g^{-1} soil in our study), the negative N effect on CO_2 flux switched and resulted in a positive relationship between CO_2 and N_2O fluxes.

4. Discussion

Through a series of laboratory experiments we found important connections between C and N biogeochemical cycles with both resources important for CO_2 and N_2O emissions. Additional C or N substrates caused an increasing soil CO_2 or N_2O flux because of more C or N resources available for decomposition. The straightforward prediction of the relationship between trace gas fluxes and its primary substrate is true, although it masks substantial contributions from coupled C and N interactions on microbial activity. Our results show that N availability can substantially influence the effect of C availability on CO_2 emissions,

and C availability can alter N_2O flux sensitivity to N addition by more than two orders of magnitude. Notably depending on the stoichiometry of resource amendments, the effects of the secondary resource could both enhance or inhibit emissions of gases. CO_2 emissions were inhibited by N at non-saturating conditions and similarly N_2O was inhibited by C at non-saturating conditions. However, saturating levels of both C and N accelerated trace gas emissions of both CO_2 and N_2O. These divergent effects of altered resources with both enhancement and inhibition suggest complex interactions between C and N biogeochemical cycles, with substantial implications for predicting emissions of GHGs.

4.1. Regulating CO_2 flux by carbon use efficiency (CUE)

High CUE is commonly observed in response to C limitation (Sinsabaugh *et al* 2013), which results in a lower respiration rate. At saturating N and sub-saturated, an increasing CUE could mobilize more C into microbes, leading to relatively lower CO_2 fluxes compared to those without N application (figures 2(a) and (b)). Owing to more N availability, C becomes limiting and soil microorganisms with a relatively fixed organismal stoichiometry require relatively more carbon for growth, which results in a higher CUE and reduced CO_2 fluxes. This dynamic CUE could explain why N addition triggered a decreased CO_2 flux. After C availability increases to a critical level, more C substrate leads to a decreasing CUE and increasing CO_2 fluxes (figure 2(a)). During N limited conditions (figure 2(c)), even when C substrate is saturating, a decreasing CO_2 flux occurs in response to relatively low levels of N addition. Exposed to an excess C source and restricted in growth by N, the microorganisms may adjust their metabolism, i.e., uncoupling catabolism and anabolism via energy spilling pathways associated with decreased CUE (Sinsabaugh *et al* 2013, Gallmetzer and Burgstaller 2002, Vrabl *et al* 2009, Larsson *et al* 1995). However, N amendment will alleviate N limitation and increase CUE. When N is not limiting, the microbes coupled catabolism and anabolism again and the excess C source will introduce a higher CO_2 flux associated with lower CUE (figure 2(c) with dextrose).

Thus, our results support a hypothesis of dynamic CUE that can explain CO_2 flux response to C and N additions. However, the mechanism for soil microbial changes in metabolic pathway under different resource environments that allow adjustment of CUE is unclear and should be targeted for future research. Nevertheless, process models that incorporate a dynamic CUE to estimate CO_2 flux seem warranted.
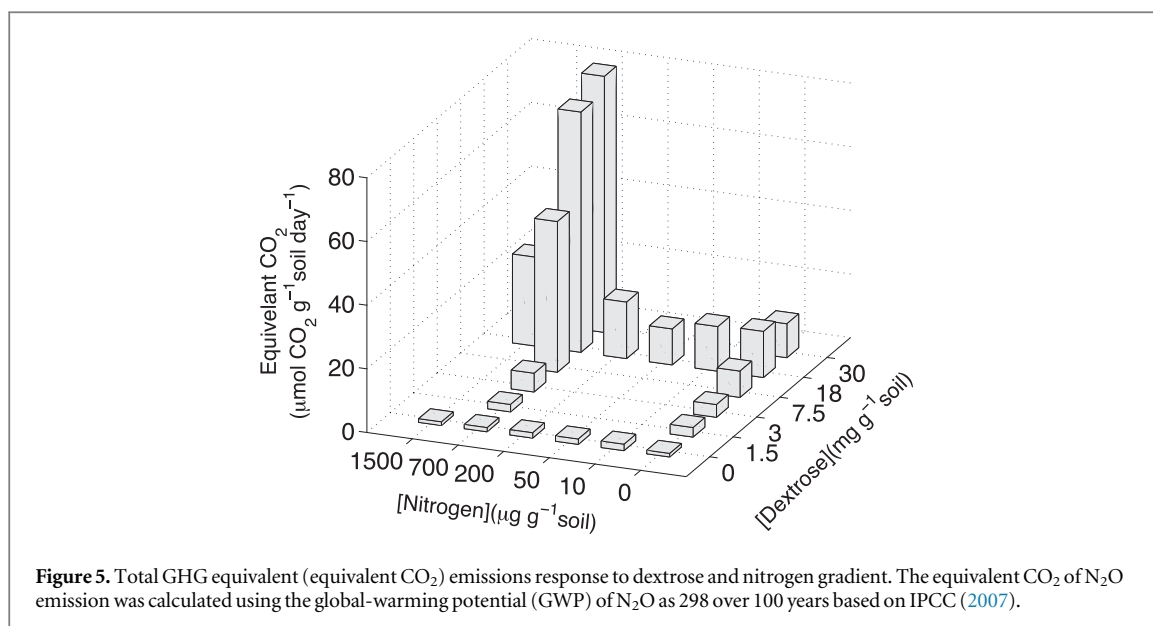
4.2. Regulating N_2O flux

From ecological stoichiometry, a higher NUE (related to a lower N_2O flux) could be expected under N limitation (Mooshammer *et al* 2014, Sterner and Elser 2002), which implies the limited N would be conserved primarily for growth. The decreasing N_2O flux associated with low rates of extra C source (figure 3(a) no nitrogen; figure 3(b) with dextrose in lower N levels) suggests that more N has been used to build soil microbial biomass as the extra C is also distributed into growth, which results in a higher NUE and lower N_2O flux. Alternatively, exogenous C source provides additional electrons (i.e., NADH) via carbon degradation pathways and the TCA cycle to reduce the N_2O to N_2 by denitrifying enzymes (Giles *et al* 2012, Richardson 2000). Regardless of how N_2O is generated from the N cycle pathways (either nitrification or denitrification) (Butterbach-Bahl *et al* 2013), NADH promotes reduction of N_2O into N_2 via the electron transport chain. Such a reduction in the $N_2O:N_2$ ratio in response to labile carbon substrates has been shown (Morley and Baggs 2010, Giles *et al* 2012, Lee and Jose 2003, Weier *et al* 1993), although the magnitude varies because of the divergence in C substrate quality, soil conditions and O_2 availability (Morley and Baggs 2010, Giles *et al* 2012, Lee and Jose 2003).

N_2O flux responses to additional C source under limiting N conditions may be regulated by NUE or the interaction between carbon and nitrogen metabolism through nitrification or denitrification pathways. As with CO_2 emissions, while the mechanism for variable NUE is unclear, these findings support the need for improvements in process models that account for resource stoichiometry and C and N interactions rather than N availability alone to estimate N_2O emissions (Liu *et al* 2012, Jassal *et al* 2011).

4.3. Coupled CO_2 and N_2O flux relationships

Availabilities of C and N substrates simultaneously regulate CO_2 and N_2O fluxes. From our results, the relationship between soil CO_2 and N_2O fluxes can be switched from negative to positive (figure 4) based on the N supplement. The critical level for this switch might be a result of the switch in elemental requirement from C to N for microorganism growth. A threshold elemental ratio (TER), which is a parameter in quantifying when growth limitation switches from one element to another (Frost *et al* 2006, Sterner and Hessen 1994), can control the metabolism of microorganisms (Mooshammer *et al* 2014). If the C:N ratio is above the TER, the metabolism of soil microbial communities is under N limitation and expresses a relatively higher NUE but lower CUE. The negative relationship between CO_2 and N_2O fluxes occurs (figure 4 and figures 2(c) and 3(b) when N is low). In contrast, an expected lower NUE but higher CUE would occur when the C:N ratio is below the TER, which is a C limiting condition. The negative relationship still occurs between CO_2 and N_2O when N is at the control level (figure 4 and figures 2(a) and 3(a) no nitrogen). When C and N availability are both

Figure 5. Total GHG equivalent (equivalent CO_2) emissions response to dextrose and nitrogen gradient. The equivalent CO_2 of N_2O emission was calculated using the global-warming potential (GWP) of N_2O as 298 over 100 years based on IPCC (2007).

available at high levels, elevated CO_2 and N_2O fluxes will be produced and a positive relationship between them is seen (figure 4). Based on the contrary relationship between CO_2 and N_2O fluxes, a critical level of C:N ratio could exist in regulating the response of soil microbial CUE and NUE to substrate availability and controlling the pattern of GHG emissions.

4.4. Implications for total GHG emissions

At global scale, about 80% of N_2O emission is derived from agricultural ecosystems because of synthetic fertilizers used in agricultural soil management (Majumdar 2013, Davidson 2012). In high production agricultural ecosystem, a large amount of carbon substrate can be introduced into the soil via root exudation or residues (Oikawa *et al* 2014). These substrates have a large influence on CO_2 emissions and as suggested here may also influence N_2O fluxes. Similarly, our results suggest patterns of fertilization may also have direct effects on CO_2 emissions. With N_2O having a much higher warming potential (298 times that of CO_2 over 100 years) (IPCC 2007) than CO_2, these interactions between C and N biogeochemical cycles may have important consequences for net emissions (figure 5). Without additional N, total GHG emissions increased linearly with C additions, while without additional C, total GHG emissions increased minimally with N additions. The largest increases occurred when both C and N were added and total GHG emissions were ~70, ~5 and ~60 times higher than the control samples, C or N amendments, respectively. Extending these findings to the field is a clear research need for understanding how soil emissions of both CO_2 and N_2O contribute to total warming potential in response to coupling between N and C cycles. The divergent effects from limited C or N on CO_2 and N_2O fluxes result in an opposite

relationship between them, suggesting the possibility to minimize total GHG emissions by optimizing fertilizer level and timing relative to growth in agricultural management. Moreover, these results highlight the importance of C and N interactions for the ability to understand and predict GHG emissions using biogeochemical models.

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