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ORIGINAL PAPER

Oxygen and substrate availability interactively control the temperature sensitivity of CO_2 and N_2O emission from soil

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Abstract We investigated how oxygen availability, substrate amount, and quality affect the temperature dependency of enzymatic processes involved in the production of carbon dioxide (CO_2) and nitrous oxide (N_2O). Three substrates differing in microbial degradability (glucose with potassium nitrate, glycine, and phenylalanine) were added to a mountain grassland soil at a range of concentrations. Soils were incubated at 21 and 1 % of O_2 content and at 10 and 20 °C. Oxygen availability was a main factor controlling the reaction rates and temperature sensitivity of CO_2 and N_2O production. The temperature sensitivity of CO_2 production was higher under aerobic versus oxygen-limited conditions, and the opposite dependency was observed for the N_2O production. Substrate availability was a second factor affecting the temperature sensitivity of the processes leading to the production of these gases. The temperature response was reduced under substrate limitation. Apparent activation energy for aerobic CO_2 production was similar (E_a~30 kJ mol^{-1}) for tested substrates, while E_a for anaerobic N_2O production increased in the order phenylalanine<glycine<glucose+NO_3^- having values 45, 75, and 106 kJ mol^{-1}, respectively. Commonly, the temperature sensitivity of N_2O production ($2<Q_{10}<4.5$) was much higher than that for CO_2 ($Q_{10}\leq 1.5$).

Keywords Temperature sensitivity · Michaelis–Menten kinetics · CO_2 emission · Nitrous oxide emission · Glycine · Oxygen availability

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Introduction

The production of the greenhouse gases carbon dioxide (CO_2) and nitrous oxide (N_2O) during decomposition of soil organic matter (SOM) strongly depends on temperature. Therefore, increasing temperatures likely to increase soil emissions of both greenhouse gases, i.e., a positive feedback to global warming occurs (Heimann and Reichstein 2008; von Lützow and Kögel-Knabner 2009; Bond-Lamberty and Thomson 2010; Davidson and Janssens 2006; Butterbach-Bahl and Dannenmann 2011; Veraart et al. 2011). Carbon dioxide is produced in soil as a result of microbial oxidation of organic C with the highest rates under aerobic conditions. Nitrous oxide is an end-, intermediate-, or by-product of microbial conversion of organic and mineral forms of N under aerobic or anaerobic conditions in the course of nitrification, dissimilatory nitrate reduction to ammonium, denitrification, and nitrifier denitrification (Butterbach-Bahl et al. 2013; Kool et al. 2011). Nitrifier denitrification can be an important source of soil N_2O when mineral N concentrations are low and organic substances with low C/N ratios are the key substrate for microbial N transformation processes (McLain and Martens 2005). The uptake of low molecular weight organic N (mainly amino acids) by plants and microorganisms without preceding microbial ammonification is of crucial importance in

ecosystems with low N input (Schimel and Bennett 2004; Jones and Kielland 2002). However, little is known about the temperature sensitivity of these reactions and linked emission fluxes of CO_2 and N_2O.

Acceleration of CO_2 and N_2O production by temperature increase can depend both on the quality and amount of soil organic substrate (Castaldi 2000; Hartley et al. 2007; Bradford et al. 2008) and on oxygen concentrations in aerobic and anaerobic microhabitats, coexisting in the soil small scales (Castaldi 2000; Davidson et al. 2012). Thus, aerobic and anaerobic decomposition may occur simultaneously but may respond differently to temperature increase. While CO_2 production mirrors the SOM decomposition under aerobic conditions, the relationship between the decomposition of organic molecules and the evolution of CO_2 under oxygen limitation is complicated. The link between decomposition of SOM and N_2O production is not always evident since N_2O production is mediated by both biotic and abiotic reactions and by oxygen availability. Usually, N_2O-N represents a small portion of mineralized N even under anaerobic conditions and the percentage of total mineralized N present as N_2O may vary depending on substrates used by microorganisms (Butterbach-Bahl et al. 2013; Zhu et al. 2013). Therefore, the relative temperature sensitivities of CO_2 and N_2O production in soils are different under aerobic and anaerobic conditions and need to be further studied for a better understanding of how global warming might affect soil–atmosphere CO_2 and N_2O fluxes.

If soil CO_2 and N_2O production are restricted by substrate amount, the temperature sensitivity of SOM decomposition can be mainly described by the Michaelis–Menten equation (Davidson et al. 2012). It is unknown how the parameters of this equation, i.e., affinity of microbial enzyme to substrate (K_m) and heterotrophic potential (V_{max}), respond to temperature changes. As V_{max} and K_m are placed in the Michaelis–Menten equation in the numerator and the denominator, respectively, the simultaneous increase of both parameters with temperature may not result in an increase in the reaction rate at low substrate concentration, when K_m becomes significant (Davidson and Janssens 2006). This "canceling effect" was described for the mineralization of glucose in soil (Gershenson et al. 2009; Larionova et al. 2007). As CO_2 production is strongly dependent on N availability, we hypothesized that soil CO_2 production may be affected by the chemical form of available N similarly to N_2O production. In order to verify this hypothesis, we have studied the temperature sensitivity of soil N_2O and CO_2 production as affected by the substrate type and oxygen supply. We have incubated a mountain grassland soil with three substrates with different C/N ratios (glucose with potassium nitrate, glycine, and phenylalanine) under aerobic and anaerobic conditions and at two temperatures (10 and 20 °C). In this way, we have evaluated the temperature sensitivity of different metabolic routes for CO_2 and N_2O production under aerobic and anaerobic conditions. The estimation of temperature sensitivity of denitrification per se is necessary for modeling purposes and mechanistic understanding of the process.

Methods

Soil sampling

Soil cores of 16.8 cm diameter were sampled from the topsoil (0–10 cm) of five randomly chosen replicated sites at the Terrestrial Environmental Observatories (TERENO) prealpine observatory in Graswang (a Bavarian Alps mountain range in Southern Germany). The investigated soil is a C-rich Haplic Cambisol (Calcaric, Humic, Siltic) from alluvial gravel under grassland (Haplic Cambisol-G). Its main properties are the following: pH (0.01 M $CaCl_2$) 7.0; bulk density 0.8±0.1 g cm^{-3}; sand, silt, clay contents—10±1, 63±12, 27±11 %, respectively; C_{total} 142±6 mg g^{-1}, C_{org}/N_{tot} ratio 8.6. More details can be found in Unteregelsbacher et al. (2013).

Soil cores were transported to the laboratory, put together, and manually homogenized with the removal of plant debris prior to sieving (<2 mm). The soil was moistened to 40 % of the maximum water holding capacity (WHC) and divided into two parts. Then, the soil was preincubated at either 10 or 20 °C for 48 h in polyethylene bags sealed with cotton–wool plugs to allow for gas exchange.

Determination of substrate-dependent CO_2 and N_2O emission

Following pre-incubation, subsamples of soil (5 g, on oven dry basis) were weighed into 150 ml glass vials. After the addition of the substrate, the vials were flushed for 1 min with either synthetic air for aerobic incubation or with helium for micro-aerobic incubation, sealed gas-tight, and the increase in CO_2 and N_2O concentrations in the headspace of the vials was measured by syringe sampling and subsequent gas chromatographic (GC) analysis. Short-time scale (less than 15 min) and single, onefold sampling were performed according to the method requirements considering the very quick uptake of substrates by soil microorganisms (Hobbie and Hobbie 2012). Measurements of gaseous headspace in vials were also done immediately after sealing to ensure that the initial CO_2 and N_2O concentrations in the bottles after flushing were below 10 and 0.05 cm^3 m^{-3}, respectively.

The full-factorial experiment was designed with two independent factors: (1) temperature (10 and 20 °C) and (2) oxygen concentration (1 %, micro-aerobic; and 20 %, aerobic).

Two types of easily degradable substrates were used: sugar (glucose—$C_6H_{12}O_6$) and amino acids (glycine—$C_2H_5NO_2$, and phenylalanine—$C_9H_{11}NO_2$). As both glycine and phenylalanine contain N, the glucose treatment was supplemented with KNO_3. The C/N ratio of glycine (2) is much lower than

that of phenylalanine (9); therefore, the intermediate C/N ratio of 3.5 was adjusted for the glucose treatment by adding KNO_3 (glucose+nitrate). Substrate with this C/N ratio provides sufficient N for microbial immobilization and plant uptake, so that excess of N should not limit denitrification (Hodge et al. 2000; Blagodatsky and Yevdokimov 1998). Substrates were added to soil as solutions so as to bring soil moisture to 60 % WHC.

The optimal incubation period and the optimal amount of glucose required for the maximum respiratory response of soil were determined in preliminary experiments (data not shown). Glycine and phenylalanine were added to soil in the same range as C-glucose rates, i.e., 2.5–500 μg C g^{-1}. The following C substrate rates were tested: 0, 2.5, 5, 10, 25, 50, 125, 250, and 500 μg C g^{-1} (sdw).

Chemical analysis

Measurements of total C, carbonate, and N content in soil samples were performed according to DIN ISO 13878 and 10694. Colorimetrical analysis of NH_4^+ and NO_3^- in soil extracts was performed according to the VD LUFA method A 6141 (Hoffmann 1991) by a commercial laboratory (Dr. Janssen, Gillersheim, Germany). Organic C content was calculated by subtraction of carbonate-C from total C content. Soil pH was measured in 0.01 M $CaCl_2$ with a soil to solution ratio of 1:2 using a combined electrode.

Gas analysis

The concentrations of CO_2 and N_2O in the headspace of the glass vials and glass flasks were determined by gas chromatograph (PerkinElmer 8500) equipped with a thermal conductivity detector (TCD) and an electron capture detector (ECD). Measurements were conducted using a separation column GS-Q (30 m Megabore; 0.53 mm inner diameter) at the following conditions: oven temperature 40 °C, TCD temperature 250 °C, carrier gas He 5.0 at 20 ml min; or ECD temperature 350 °C, carrier gas 5 % methane 3.5 in argon 4.8 at 40 ml min^{-1}; the latter conditions were used for N_2O determination. Standard gases were used for internal calibration of the GC and for calculation of the headspace concentrations of CO_2 and N_2O.

Statistical analysis and modeling

Each treatment was replicated three times. All results are expressed as arithmetic means±standard deviation. The Michaelis–Menten kinetics (Panikov et al. 1992) was applied to describe aerobic CO_2 and anaerobic N_2O emission rate (v) as affected by the added substrate concentration (S):

$$v = V_{max} S/(K_m + S_n + S); \quad (1)$$

where V_{max} is the maximum mineralization rate or heterotrophic potential and K_m+S_n is the sum of the enzyme saturation constant and the concentration of soil indigenous C equivalent to added substrate. The parameters of Eq. (1) were fitted by minimizing the least-square sum using ModelMaker Version 3.0.3 software (ModelMaker 1997).

The Arrhenius plot ($\ln(V_{max})$ versus $1/T$) was used to calculate the apparent activation energy for CO_2 and N_2O emission at saturation concentrations of substrates, assuming a linear relationship between V_{max} and temperature in the range from 10 to 20 °C (Abdalla et al. 2009).

Results

CO_2 evolution under aerobic conditions

Soil CO_2 evolution under aerobic conditions followed the Michaelis–Menten kinetics (increasing respiration until saturation at both temperatures) for all three tested substrates (Fig. 1a–c). Both the maximal rate of substrate mineralization (V_{max}) and the affinity of enzyme systems to substrate (K_m) decreased by increasing the C/N ratio of the substrate, i.e., in the order glycine>glucose>phenylalanine (Fig. 1d, e). As a result, the absolute values of CO_2 evolution from glycine were two and three times higher, respectively, than those from glucose/nitrate and phenylalanine (Fig. 1a–c). Despite this, at substrate concentrations exceeding 100 μg C g^{-1}, the Q_{10} values (about 1.5) were similar for the three substrates (Fig. 1f). However, at substrate concentrations below K_m, the Q_{10} values decreased by decreasing substrate concentrations. A lower temperature sensitivity of glucose and glycine versus phenylalanine was observed at low substrate concentration. Furthermore, the difference between CO_2 evolution at 10 and 20 °C due to glycine and glucose additions <50 μg C g^{-1} was insignificant (Fig. 1a–c; t test gives P values for glucose and glycine of 0.055 and 0.304, respectively). The diminishing difference in CO_2 evolution rates following the addition of both substrates at low concentration was due to a higher increase in K_m than in V_{max} values by increasing temperature (Fig. 1e, f). Consequently, the $Q_{10}{}^{Km}$ exceeded the $Q_{10}{}^{Vmax}$ by a factor of 1.4 and of 3.5 for glycine and glucose, respectively (Table 1).

Values of the activation energy for CO_2 evolution from the different substrates were similar (Table 1).

CO_2 evolution under oxygen limitation

The CO_2 evolution under oxygen limitation at maximal rates of the added substrate was about 3 (glycine, glucose) to 4.5 (phenylalanine) times smaller than the respective values under aerobic conditions. Except for glycine, no clear saturation patterns were observed for CO_2 efflux by increasing substrate concentrations under oxygen limitation. Therefore, average

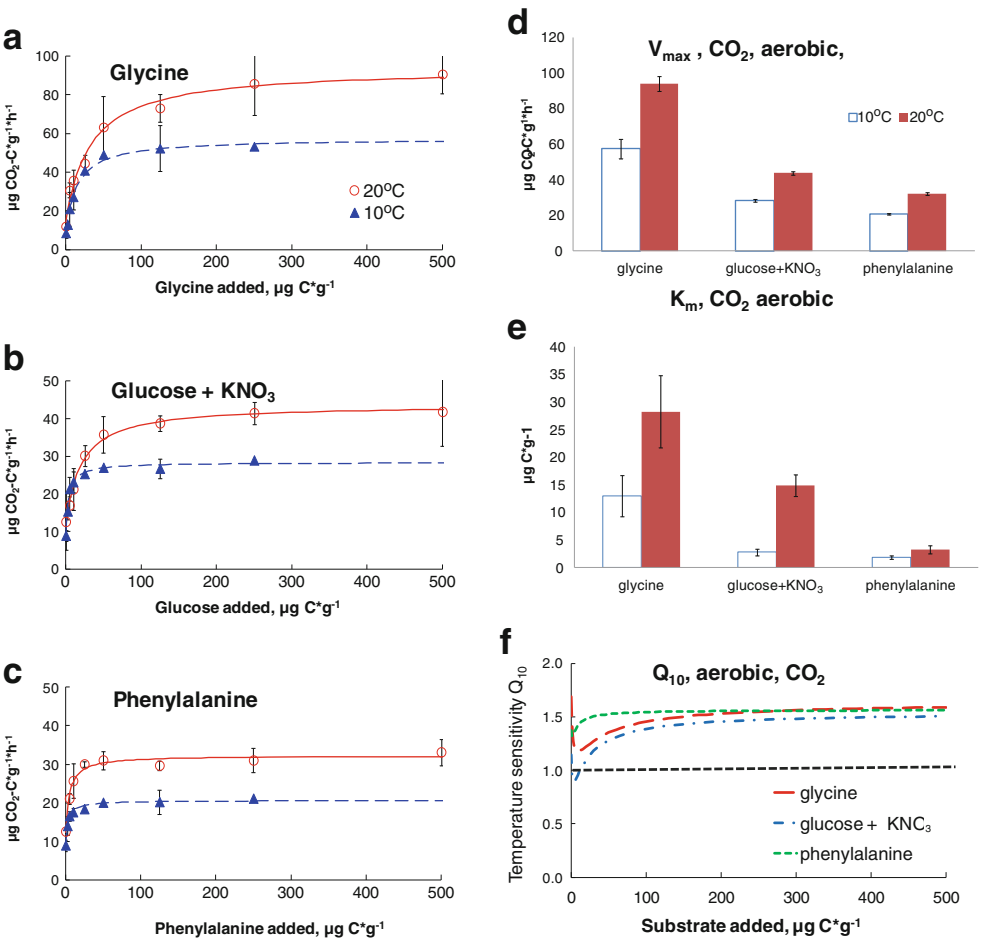

Fig. 1 Temperature effect on mineralization kinetics of glycine, glucose+KNO_3, and phenylalanine under aerobic conditions expressed as CO_2 production rate (**a**, **b**, **c**) and on values of kinetic parameters (**d**, **e**). *Bottom right graph* (**f**) shows dependence of Q_{10} from C application rate. *Symbols on left side graphs* represent experimental data (means±SE, n=3) and *curves* represent fits to the Eq. 1. Note the larger y-axes scale for glycine (**a**)

values and the range of variations for low (0–25 μg C g^{-1}) and high (50–500 μg C g^{-1}) substrate additions are presented in Table 2. Significantly higher CO_2 evolution at 20 °C than 10 °C occurred only for phenylalanine and resulted in Q_{10} varying between 1.5 and 2 (Fig. 2).

N_2O evolution under aerobic conditions

Under aerobic incubation conditions, very low rates of soil N_2O production were observed for the glucose+nitrate and phenylalanine treatments with no fit to Michaelis–Menten kinetics. However, we found significant N_2O production for the glycine treatment. Rates of N_2O production are shown as average values and as the range of variations for limiting and saturating substrate amounts (Table 3). The highest Q_{10} value (about 2) was observed for the glycine treatment at substrate additions exceeding 150 μg N g^{-1} (Fig. 3); N_2O production from the phenylalanine treatment at 10 °C was below the detection limit, so that Q_{10} values were not calculated.

Table 1 The values of Q_{10} for K_m and of Q_{10} for V_{max} and activation energy (E_a) for aerobic CO_2 emission and anaerobic N_2O emission

Substrate	Q_{10}^{Km}		Q_{10}^{Vmax}		E_a, kJ mol^{-1}	
	CO_2	N_2O	CO_2	N_2O	CO_2	N_2O
Glycine	2.17	1.59	1.64	2.43	30	75
Glucose+KNO_3	5.35	9.02	1.54	4.77	33	106
Phenylalanine	1.78	0.53	1.56	1.93	29	45

N_2O evolution under oxygen limitation

For all three substrates, the substrate-dependent patterns of N_2O evolution under anaerobic conditions followed Michaelis–Menten kinetics (Fig. 4a–c). At high substrate addition rates (50–300 μg N g^{-1}), the temperature response of N_2O evolution for glucose plus nitrate was twice as high as those due to glycine and phenylalanine additions (Fig. 4f). The Q_{10} values for N_2O evolution in the glucose plus nitrate treatment decreased at N addition rates below 10–14 μg N g^{-1} and became even smaller than those from glycine and phenylalanine treatments. This was due to an almost doubled temperature sensitivity of the K_m versus V_{max} in the glucose plus nitrate treatment (Table 1). The Q_{10} values for N_2O evolution were smaller for glucose plus nitrate than those due to glycine

Table 2 Average values and the range of variations of anaerobic CO_2 emission rates for small (0–25 μg C g^{-1}) and large (50–500 μg C g^{-1}) substrate amounts

Substrate	Temperature	Substrate applied at a rate:			
		0–25 μg C g^{-1}		50–500 μg C g^{-1}	
	°C	Average μg C g^{-1} h^{-1}	Range	Average	Range
Glycine	10	13.9	4.2–23.4	29.0	27.1–31.8
	20	10.6	7.2–15.3	25.5	19.0–31.5
Glucose+KNO_3	10	9.9	3.9–14.2	9.6	8.7–10.4
	20	10.5	7.8–8.8	6.2	4.9–7.7
Phenylalanine	10	3.91	3.52–4.36	3.81	3.59–4.13
	20	6.55	6.17–7.14	6.47	6.07–6.88

and phenylalanine additions when rates were lower than 15 μg N g^{-1}. On the contrary, the Q_{10} for N_2O evolution was almost twofold for glucose plus nitrate than for the glycine and phenylalanine treatments at saturating substrate concentrations. The Q_{10} for V_{max} was always higher than the Q_{10} for K_m for the glycine and phenylalanine treatments. In contrast to the activation energy for aerobic CO_2, the energy (E_a) values for anaerobic N_2O emission were lower by a factor of 1.6 and 2.4 for phenylalanine than for glycine and for glucose plus nitrate, respectively (Table 1).

Discussion

Temperature sensitivity of CO_2 and N_2O evolution as affected by oxygen availability

Under anoxic conditions, the CO_2 evolution was limited both by substrate availability and by O_2 availability. Due to O_2 limitation, a substrate addition effect on CO_2 evolution could hardly be detected in our study. Much smaller anaerobic versus aerobic CO_2 efflux indicated differences in metabolic pathways with slower process rates under oxygen limited versus aerobic conditions (Paul 2007). The oxygen limitation did not only affect the CO_2 evolution rates during the decomposition of tested substrates but also masked the response of the decomposition rate to the temperature increase and did not allow calculating a

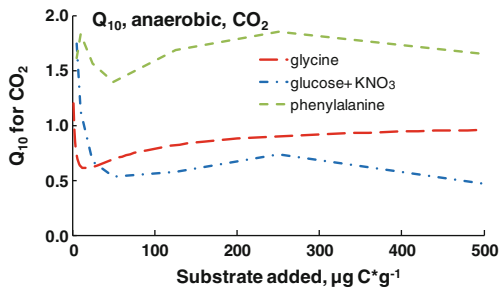

Fig. 2 Q_{10} for CO_2 production rate under oxygen-limited conditions as affected by C application rate of glycine, glucose+KNO_3, and phenylalanine

relationships between substrate concentration and Q_{10}. Therefore, the oxygen availability or degree of anaerobiosis needs to be considered before quantifying temperature effect on decomposition and CO_2 evolution (Davidson et al. 2012).

Varying patterns and amounts of evolved N_2O with glucose+nitrate, glycine, and phenylalanine addition under oxygen-limited conditions reflected the substrate limitation of processes involved in N_2O evolution. Under oxygen limitation, nitrate serves as electron acceptor for denitrification in the glucose plus nitrate treatment. When nitrate is limited but N is present as glycine (C/N ratio of 2), both heterotrophic nitrification and denitrification may occur in same microbial cell (Blagodatsky et al. 2006).

For all substrates tested, the N_2O efflux under oxygen limitation was more temperature sensitive (Q_{10} 2–4.5) than the aerobic CO_2 emission ($Q_{10} \leq 1.5$). This observation corresponds to studies which found that the temperature sensitivity of conventional denitrification under anaerobic conditions and excess of substrates (nitrates and C as energy source) was higher than the temperature sensitivity of CO_2 evolution from aerobic decomposition of SOM (Holtan-Hartwig et al. 2002). A similar conclusion was made in the study of Abdalla et al. (2009), who also found that the Q_{10} values for N_2O evolution ranged from 4.4 to 6.2, i.e., they were higher than the Q_{10} for typical biochemical reactions. The possible explanation of this phenomenon could be the increased anaerobiosis in soil due to increased microbial respiration at elevated temperatures (Smith 1997; Oquist et al. 2004; Butterbach-Bahl and Dannenmann 2011). Thus, despite low absolute values of N_2O evolution as compared to CO_2 evolution, the contribution of N_2O to the temperature-driven acceleration of the greenhouse effect may be substantial and thus needs to be considered in future global change scenarios.

Effect of substrate availability on temperature sensitivity of CO_2 and N_2O evolution

The Q_{10} values observed in this study (about 1.5) for immediate aerobic soil CO_2 evolution in response to the addition of

Table 3 Average values and the range of variations of aerobic N_2O emission rates for small (0–14.6 μg N g^{-1}) and large (36.5–146.2 μg N g^{-1}) substrate amounts

Substrate	Temperature	Substrate applied at a rate:			
		0–14.6 μg N g^{-1}		36.5–146.2 μg N g^{-1}	
	°C	Average μg N g^{-1} h^{-1}	Range	Average	Range
Glycine	10	0.06	0.09–0.13	0.21	0.19–0.23
	20	0.07	0.02–0.15	0.35	0.24–0.44
Glucose+KNO_3	10	0.025	0.012–0.046	0.022	0.018–0.026
	20	0.066	0.013–0.119	0.034	0.028–0.041
Phenylalanine	10	ND[a]	ND	ND	ND
	20	0.95	0.07–1.42	0.14	0.01–0.52

[a] ND not determined, as N_2O production from phenylalanine treatment at 10 °C was below detection limit

easily available organic substrate at saturating concentration were at the lower values of the Q_{10} range obtained in short-term laboratory incubations (1.9–2.2, Castaldi 2000; 2.0, Vinolas et al. 2001; 1.4–3.3, Hopkins et al. 2006). Our Q_{10} values of anaerobic N_2O evolution of 1.9–4.5 confirm the range of values (1.7 to 9.3) by Abdalla et al. (2009). The activation energy for N_2O evolution from phenylalanine (45 kJ mol^{-1}) was similar to that calculated for grassland pasture (47 kJ mol^{-1}, Abdalla et al. 2009), while the E_a for glucose plus nitrate and glycine treatments were in the range of 75 to 106 kJ mol^{-1}.

Carbon dioxide efflux showed that at both temperatures the potential decomposition rates were always higher for glycine than for phenylalanine or glucose plus nitrate treatments confirming already published data (O'Down and Hopkins 1998; Hartley et al. 2010). So, glycine combines C and N source with a ratio more suitable for microbial assimilation as compared to phenylalanine. Amino acids can be directly included in biosynthesis, while nitrates need to be reduced to amides and only afterwards the N is incorporated in proteins. Therefore, lower rates of CO_2 evolution were observed in glucose plus nitrate than in glycine treatment.

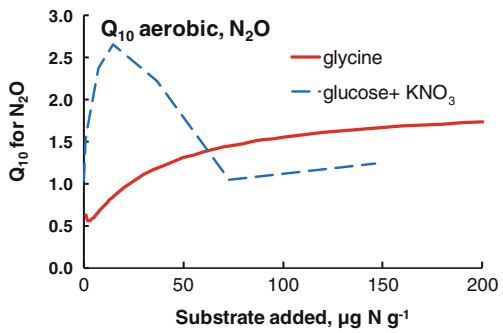

Fig. 3 Q_{10} for N_2O emission rate under aerobic conditions as affected by the N application rate of glycine and glucose+KNO_3. N_2O production from phenylalanine treatment at 10 °C was below detection limit; therefore, Q_{10} values were not estimated in this case

The observed reduction of the temperature sensitivity of aerobic CO_2 evolution at lower rates of substrate additions demonstrates that substrate availability obviously controls the temperature sensitivity of organic matter decomposition without oxygen or nitrogen supply as limiting factors. This confirms the hypotheses by Kirschbaum (2006), who also found a reduced temperature sensitivity of organic matter decomposition after depletion of the pool of labile substrate. We found no differences in substrate-induced CO_2 evolution rates between 10 and 20 °C if only small amounts ($<K_m$) of glycine or glucose plus nitrate were added. However, the phenylalanine decomposition was temperature sensitive even at low substrate concentration, indicating a smaller "canceling effect" for phenylalanine due to its lowest K_m (Fig. 1e, f). The temperature sensitivity of soil CO_2 evolution under aerobic conditions was similar among the tested substrates at saturating substrate concentrations. This indicates that decomposition of any added substrate was not limited by N availability. Hence, we suggest that a holistic perspective of the temperature sensitivity of C mineralization inevitably needs to consider substrate availability, i.e., the interactive effect of substrate amount and quality.

Effect of organic and mineral forms of N on N_2O evolution at different temperatures

Anaerobic N_2O evolution at 20 °C was higher for the glucose plus nitrate treatment than for glycine-amended soil, whereas for the phenylalanine treatment, the lowest N_2O evolution rates were observed. Nitrogen from organic substrates with a wide C/N ratio as phenylalanine (C/N=9) is preferably immobilized into microbial biomass rather than ammonified and/or nitrified and subsequently used within the denitrification chain as electron acceptors. On the contrary, the oxidized form of N in nitrates (as seen for the glucose plus nitrate treatment) can be easily used for denitrification under

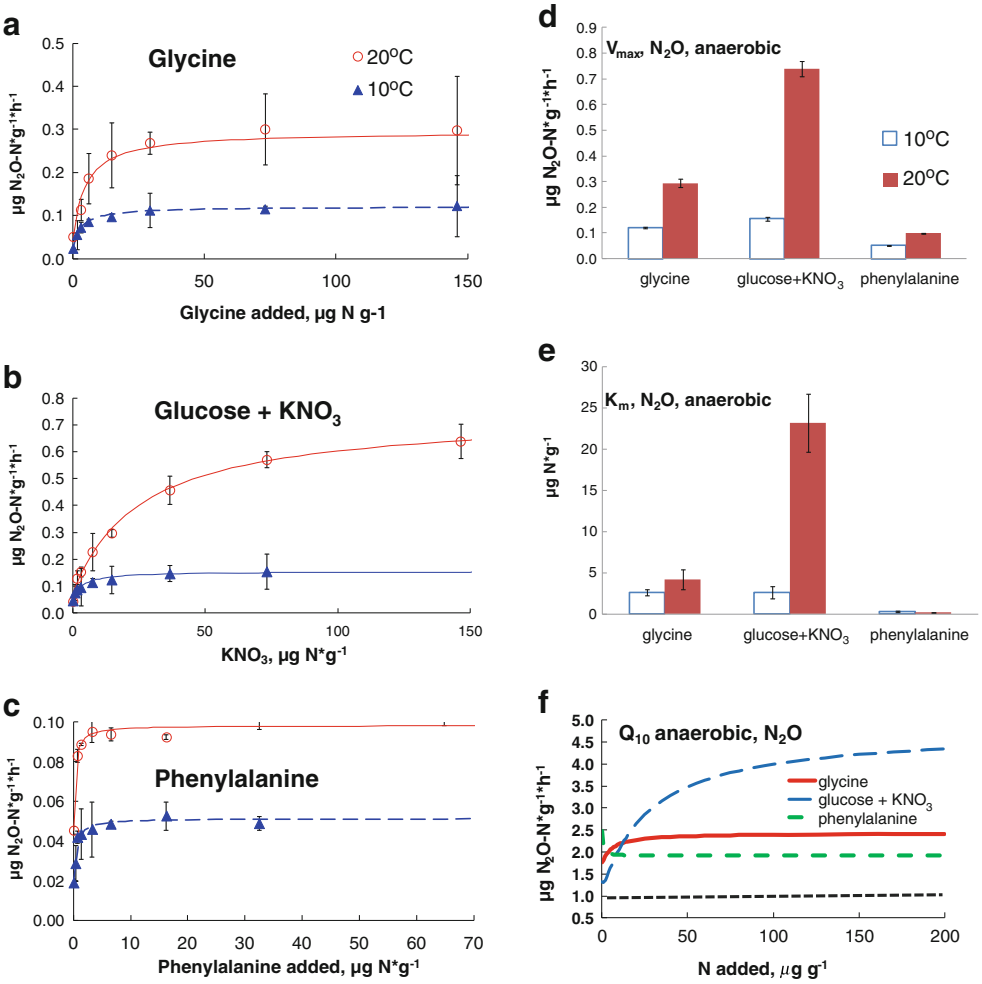

Fig. 4 Temperature effect on N_2O emission rate during mineralization of glycine, glucose+KNO_3, and phenylalanine applied at different concentrations under oxygen limiting conditions (*left* panel) and on values of kinetic parameters (*right top* and *middle*). *Bottom right graph* shows dependence of Q_{10} on N application rate. *Symbols on left side graphs* represent experimental data (means±SE, n=3) and *curves* represent fits to the Eq. 1. Note different scales for three applied substrates

anaerobic conditions, such that high rates of N_2O evolution by denitrification occur. Future research should compare glucose plus nitrate and glucose plus ammonium treatments to complete the picture and support the made conclusions concerning the prevailing denitrification route under anaerobic conditions.

The K_m was more temperature sensitive than the V_{max} for N_2O evolved under anaerobic conditions if mineral N was applied (Fig. 4d, e). The V_{max} was more temperature sensitive than the K_m in treatments with organic N sources. This finding indicates that different enzyme systems are involved in transformations of mineral and organic forms of N (Khalili et al. 2011; Davidson et al. 2012). Remarkably, the differences between treatments in temperature response of N_2O evolution were immediate (within 10 to 15 min) after the soil became anaerobic. As all denitrifying enzymes were found to be active in the soil at the onset of anaerobiosis (Holtan-Hartwig et al. 2002), the different K_m values indicated inherent differences in the regulatory pathways in the denitrification gene expression between microbial groups growing on different substrates, i.e., glucose versus glycine (Braker et al. 2012).

The temperature sensitivity of anaerobic N_2O evolution was dependent on both substrate quality and amount (Fig. 4f). The double limitation of denitrification by energy supply (i.e., by reduced organic C) and by electron acceptors (i.e., oxidized forms of N) helps to explain the observed dependencies. The rate of anaerobic N_2O evolution in glycine-amended soil reached the saturation level (~0.3 µg N g^{-1} soil h^{-1}) at a lower concentration of supplied substrate as compared to glucose- and nitrate-amended soil (Fig. 4a, b). The N_2O evolution rate did not increase when the amount of N added as glycine exceeded the ambient nitrate content in soil used in the experiment (22.8 µg N g^{-1} soil, STD=1.25). It therefore seems that nitrate concentration was limiting N_2O emission in glycine-amended soil and that glycine mainly functioned as a C source and reducing equivalents for denitrification, similar to the glutamic acid addition experiment carried out by Holtan-Hartwig et al. (2000). The saturation level for N_2O evolution (apparent V_{max}) for phenylalanine was reached at N concentration lower than 5 µg N g^{-1} soil. This means that in this case denitrification was limited by reduced organic C source and not by nitrate concentrations. Phenylalanine could not serve as an appropriate carbon source for

denitrification during short-term incubations as in our study. This also explains the insensitivity of Q_{10} dependency on applied phenylalanine amounts (Fig. 4c).

Surprisingly, greater N_2O evolution was observed in glycine treatment under aerobic as compared with anaerobic conditions at saturation substrate concentrations. In contrast to the oxygen limiting conditions, the N_2O evolution rate was not limited by indigenous soil nitrate concentration and increased with the amount of organic N applied—up to 250 μg N g^{-1} soil, i.e., much larger the nitrate N content in soil. Probably heterotrophic nitrification combined with aerobic denitrification was the route for the N_2O evolution from amino acids. This finding calls for further studies of temperature effect on N_2O emission under aerobic versus anaerobic conditions.

Conclusion

We found that oxygen availability is of crucial importance in regulating the temperature sensitivity of soil CO_2 and N_2O evolution. The oxygen limitation masked the response of CO_2 emission to the temperature increase, but increased the temperature sensitivity of N_2O evolution. Therefore, a careful consideration of aerobic versus anaerobic conditions is an indispensable prerequisite to better predict temperature effects on decomposition and emission of greenhouse gases. The other factors, i.e., available substrate concentration and substrate quality (e.g. organic versus mineral form and C/N ratio) will influence the temperature response of C and N mineralization and gas formation in the second turn, and must be considered jointly with O_2 limitation. Similar rates of N_2O evolution were observed in glycine and glucose plus nitrate treatments at 10 °C. Substantially larger temperature sensitivity of N_2O evolution from glucose plus nitrate than from the investigated organic N forms (phenylalanine, glycine) indicated different biochemical pathways and underlined the importance of the organic pathway of N_2O evolution at low temperatures. The results of current study imply that temperature correction factors for greenhouse gases emission should be included in the ecosystem models only after careful consideration of factors controlling the processes namely oxygen and substrate supply. Otherwise generalization of temperature response functions can lead to erroneous predictions by model.

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