Biol Fertil Soils (2000) 32:67–72

ORIGINAL PAPER

Simona Castaldi

Responses of nitrous oxide, dinitrogen and carbon dioxide production and oxygen consumption to temperature in forest and agricultural light-textured soils determined by model experiment

Received: 26 August 1998

Abstract The experiment, carried out on a forest and arable light-textured soil, was designed to study the temperature response of autotrophic and heterotrophic N_2O production and investigate how the N_2O flux relates to soil respiration and O_2 consumption. Although N_2O production seemed to be stimulated by a temperature increase in both soils, the relationship between production rate and temperature was different in the two soils. This seemed to depend on the different contribution of nitrification and denitrification to the overall N_2O flux. In the forest soil, almost all N_2O was derived from nitrification, and its production rate rose linearly from 2 °C to 40 °C. A stronger effect of temperature on N_2O production was observed in the arable soil, apparently as a result of an incremental contribution of denitrification to the overall N_2O flux with rising temperature. The soil respiration rate increased exponentially with temperature and was significantly correlated with N_2O production. O_2 consumption stimulated denitrification in both soils. In the arable soil, N_2O and N_2 production increased exponentially with decreasing O_2 concentration, though N_2O was the main gas produced at any temperature. In the forest soil, only the N_2 flux was related exponentially to O_2 consumption and it outweighed the rate of N_2O production only at >34 °C. Thus, it appears that in the forest soil, where nitrification was the main source of N_2O, temperature affected the N_2O flux less dramatically than in the arable soil, where a temperature increase strongly stimulated N_2O production by enhancing favourable conditions for denitrification.

Key words Denitrification · Nitrification · Nitrous oxide · Respiration · Temperature

S. Castaldi (✉)
Dipartimento di Scienze Ambientali, Seconda Università di Napoli, via Vivaldi 43, 81100 Caserta, Italy
e-mail: nw0011@mbox.netway.it
Tel.: +39-823-274646
Fax: +39-823-274506

Introduction

Temperature, together with moisture, is one of the most influential environmental factors affecting the rate of nutrient cycling and the production of greenhouse gases in soil (Kirschbaum 1995; Smith 1997). In periods when water is non-limiting (mainly in temperate zones), the processes of decomposition, mineralization and CO_2 production appear to be strongly stimulated by temperature increases (Peterjohn et al. 1993; Kirschbaum 1995; Winkler et al. 1996), and, the enhanced mineralization seems to increase the emissions of N_2O and NO (Matson et al. 1989). However, a direct effect of temperature has also been observed on N_2O production. Although this generally increases with rising temperature (Granli and Bøckman 1994; Smith 1997), the relationship between N_2O fluxes and temperature increase is not straightforward, as many different processes are involved. In fact, N_2O is produced during nitrification and denitrification, but can also be consumed by the latter. Moreover, when a temperature rise stimulates microbial respiration, i.e. O_2 consumption, the volume of the anaerobic fraction of the soil increases, enhancing denitrification activity (Parkin and Tiedje 1984; Smith 1997).

In order to make the correct assumptions to model the effect of temperature changes on the production of greenhouse gases it is important to gain more information on the response to temperature changes of soil microbial processes involved in the production of these gases. This is particularly important considering that the rise in the mean global surface temperature, due to the increased atmospheric concentration of greenhouse gases, is expected to have a positive feedback effect on the biogenic gases emissions (IPCC 1990, 1995; Smith 1997).

The present study was designed to investigate the effect of temperature changes on soil microbial processes involved in N_2O emissions. A model experiment was carried out where soils were incubated at different temperatures, and the rates of autotrophic and hetero-

trophic N_2O production, denitrification, microbial respiration and O_2 consumption were measured. Attention was focused on light-textured soils which facilitated the study of N_2O production by processes of both nitrification and denitrification, as compared with heavy textured soils where denitrification represents the only source of N_2O, especially at high temperatures.

Materials and methods

Soils used and experimental design

Two light-textured soils, a sandy loam and a loamy sand, were sampled at the beginning of April from a deciduous woodland and a nearby winter wheat field, at Gullane, East Lothian, Scotland (Castaldi and Smith 1998). Several samples were taken randomly from the top 10 cm of soil after having removed the litter layer. At the time of sampling the soil temperature was 6 °C in the forest site and 9 °C in the arable site. On the same day, soils were sieved, extracted for 1 h with 1 M KCl (100 ml 20 g^{-1} fresh soil) for the determination of available mineral N and weighed for the determination of the gravimetric water content. The remaining soil was stored overnight at 7 °C. The next day, 27 samples (50 g fresh soil) of each soil were weighed into 0.5-l conical flasks; water was added to bring the soil to 60% of water-filled pore space (WFPS), and the flasks were firmly capped and pre-incubated. Nine flasks of each soil were pre-incubated at 2 °C, nine at 25 °C and nine at 40 °C, in order to cover a wide range of temperature, from just above freezing point (0 °C) to just below the limit of protein denaturation (40 °C). However, both soils rarely presented an average monthly maximum temperature >20 °C. Ten days of pre-incubation were given to allow the microbial populations to adapt to the different temperatures. After this period the flasks were opened, flushed with synthetic air and amended with 0.2 mg NH_4NO_3 g^{-1} dry soil and 0.37 mg glucose g^{-1} dry soil using talc as a carrier (5 mg g^{-1} dry soil). The water content was adjusted to obtain 60% of WFPS. The flasks were capped with air-tight rubber lids. A needle was inserted in the rubber to allow air escape and was removed immediately after closure in order to avoid over-pressure in the flasks due to the insertion of the lids. For each series of nine flasks, three were used as controls, three were amended with 0.5 ml acetylene (0.1% C_2H_2 v/v) to inhibit the activity of the ammonia-monooxygenase (Hynes and Knowles 1978), and three were amended with 50 ml acetylene (0.1% C_2H_2 v/v), to inhibit the activity of the nitrous oxide reductase (Yoshinari and Knowles 1976). Both C_2H_2 concentrations were found optimal in preliminary experiments. The flasks were incubated for 24 h, each lot at the same temperature as in the pre-incubation. After 24 h, two consecutive 1 ml gas samples were taken using air-tight syringes. The samples were immediately analysed by gas chromatography. Autotrophic and heterotrophic N_2O production and denitrification N_2 production were calculated using the acetylene block technique results as described by Davidson et al. (1986).

Analyses

Available N was determined on the 1 M KCl extract by automated colorimetric analysis based on methods by Crooke and Simpson (1971) and Best (1976) for NH_4^+-N and NO_3^--N, respectively. The relationship between the water content and the WFPS was obtained using a measured bulk density of 1.32 for the arable and 1.15 for the forest soil. CO_2 and O_2 gas samples were measured with a gas chromatograph equipped with a thermal conductivity detector and Porapak Q packed columns. N_2O gas samples were measured with a gas chromatograph equipped with an electron capture detector (Castaldi and Smith 1998).

Results

When the temperature was raised from 2 °C to 40 °C, the average N_2O production increased from 0.2 to 5.9 ng N g^{-1} h^{-1} in the forest soil and from 0.2 to 5 ng N g^{-1} h^{-1} in the arable soil. Although the range of N_2O production was comparable, nitrification and denitrification contributed differently to the overall N_2O flux in the two soils. In the forest soil, N_2O production was derived almost completely from nitrification, and its production rate increased linearly with temperature (Fig. 1). Whereas in the arable soil an exponential relationship was found for both autotrophic and heterotrophic N_2O production (Fig. 2), and the relative contribution of denitrification was observed to increased with temperature. In both soils N_2 production rates rose exponentially with temperature in accordance with an Arrhenius equation (Fig. 1, small box; Fig. 2). However, the increase in the N_2 production rate per 10 °C increase in temperature, i.e. the Q_{10}, was much lower in the forest soil than in the arable soil (Table 1).

Above 2 °C, CO_2 production rates were significantly higher in the forest soil than in the arable soil (Fig. 3a). In both cases, the relationship between respiration rates and temperature was described by an Arrhenius equation (Fig. 3b) and the Q_{10}s calculated for the two soils were comparable (Table 1).

In both soils O_2 uptake rates, measured as O_2 disappearance rates in the jars' headspace, increased linearly with temperature, but at a significantly higher rate in the forest soil (Fig. 4). Since O_2 concentration regulates denitrification activity and the ratio N_2O/N_2, O_2 consumption rate was plotted against rates of heterotrophic N_2O and N_2 production. In the forest soil the N_2O flux increased linearly with O_2 consumption up to 30 μg O_2 g^{-1} h^{-1} and then started to slow down. In contrast, a significant exponential relationship was evi-

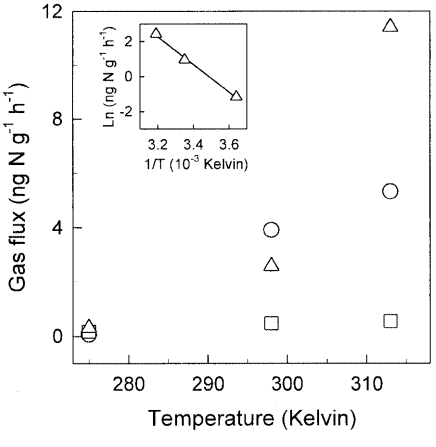

Fig. 1 Mean rates of autotrophic N_2O production(○), heterotrophic N_2O production (□) and N_2 production (△) in the forest soil samples at 2 °C, 25 °C and 40 °C. In the *upper left-hand corner* is the Arrhenius plot of the N_2 production rate. The line was fitted to data by linear regression

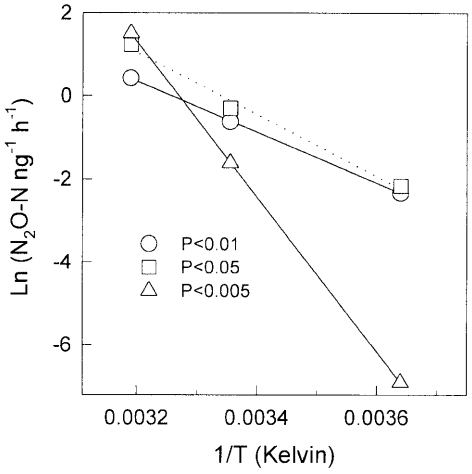

Fig. 2 Arrhenius plot of the mean rates of autotrophic N_2O production (○), heterotrophic N_2O production (□) and N_2 production (△) in the arable soil samples incubated at 2 °C, 25 °C and 40 °C. Lines were fitted to data by linear regression

denced between rates of N_2 production and O_2 consumption ($P=0.002$; Fig. 5). However, only when the latter was around 58 μg O_2 g^{-1} h^{-1} did the N_2 flux rise steeply. This O_2 consumption rate would occur at 34 °C, as calculated from the regression equation (O_2 consumption rate = 4.43 + 1.58 temperature) shown in Fig. 4.

No statistically significant relationship was evidenced between either N_2O or N_2 and O_2 consumption in the arable soil when all the replicates were plotted. However, by plotting the averages, it could be observed that autotrophic, heterotrophic and total N_2O production, as well as N_2 production, increased exponentially with O_2 consumption (Fig. 6). The steepest rise was recorded above 32 μg O_2 g^{-1} h^{-1}, which would occur at about 15 °C, as calculated from the regression equation (O_2 consumption rate = 11.2 + 0.75 temperature; Fig. 4).

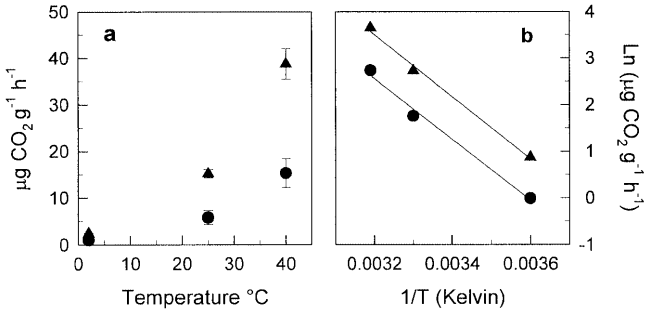

Fig. 3 Mean CO_2 emissions rates from the forest (▲) and the arable (●) soil samples at 2 °C, 25 °C and 40 °C (**a**), and the same values reported in an Arrhenius plot (**b**). *Bars* represent 1 SE. *Lines* were fitted to data by linear regression

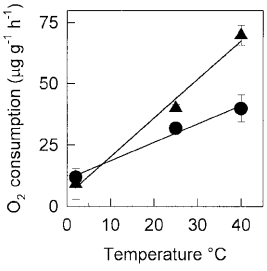

Fig. 4 Mean rates of O_2 consumption in the forest soil (▲) and in the arable soil samples (●) incubated at 2 °C, 25 °C and 40 °C. *Bars* represent 1 SE. Lines were fitted to data by linear regression

Discussion

Response of N_2O and CO_2 production to temperature in the two soils

A low N_2O flux was measured in both soils at low temperature (2 °C), in accordance with previous findings during field experiments (Castaldi 1997). Similar results were also obtained for different soils by Malhi et al. (1990) and by Dorland and Beauchamp (1991). Significant denitrification rates and N_2O fluxes have been found, indeed, to occur at low temperatures only when soil is at field capacity, because the low soil gas diffusivity under these conditions facilitates the creation of anaerobic microsites where denitrification takes place (Powlson et al. 1988).

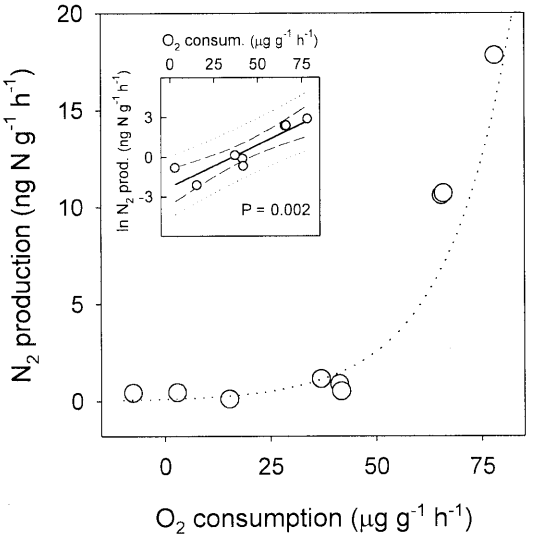

Fig. 5 N_2 production rate as a function of the O_2 consumption rate in the forest soil samples. *Dotted lines* represent a non-linear regression of the data. In the *small box* N_2 production data are reported in logarithmic scale and were fitted by linear regression. Confidence and prediction intervals are represented as *dashed and dotted lines*, respectively

Table 1 Q_{10}s calculated for the rates of autotrophic and heterotrophic N_2O production, N_2 production and CO_2 production in the arable and in the forest soil, in the temperature intervals of 2–25 °C and 25–40 °C

	Q_{10}s for production rates in the temperature ranges	
	2–25 °C	25–40 °C
Arable soil samples		
Autotrophic N_2O production	2.1	2.0
Heterotrophic N_2O production	2.0	3.4
N_2 produced by denitrification	8.9	9.7
CO_2 production	2.1	1.9
Forest soil samples		
N_2 produced by denitrification	2.5	2.7
CO_2 production	2.2	1.9

When the temperature was raised from 2 °C to 40 °C, N_2O production increased significantly. Thus, even if the microbial population was adapted to soil temperatures of <15 °C for most of the year, it responded positively to significant temperature increases.

Although the range of N_2O production was comparable, the response of autotrophic and heterotrophic N_2O production to temperature changes was different in the two soils. In the arable soil the N_2O flux had both an autotrophic and heterotrophic origin, but the relative contribution of denitrification activity increased with a rise in temperature, increasing from 53% at 2 °C to 70% at 40 °C. This might be explained by the observation that denitrification has a higher optimum temperature range than nitrification (see Granli and Bøckman 1994). However, denitrification activity might also be indirectly stimulated by a rise in temperature, which, by enhancing soil respiration and O_2 consumption, might increase the volume of the anaerobic fraction of the soil, strongly and positively correlated with denitrification activity (Parkin and Tiedje 1984; Smith 1997). This is in accordance with the observed Q_{10}s. In fact, autotrophic activity presented a Q_{10} of about 2, typical of biological reactions, while heterotrophic N_2O production presented a Q_{10} of 3.4 and a Q_{10} between 8.9 and 9.7 was calculated for N_2 production. Although a Q_{10} as low as 0.9 has been measured for N_2O emissions in laboratory experiments, high Q_{10}s have been mainly observed in association with denitrification activity (see Smith 1997). The reason for this is that a temperature rise enhances the activity of denitrifiers within the soil anaerobic microsites, and increases the volume of the anaerobic soil fraction, where denitrification occurs. Thus, when this increase is multiplied by the Q_{10} of the denitrification reaction rate *per unit volume* in the anaerobic zone, large Q_{10}s are obtained for denitrification and heterotrophic N_2O production (Smith 1997).

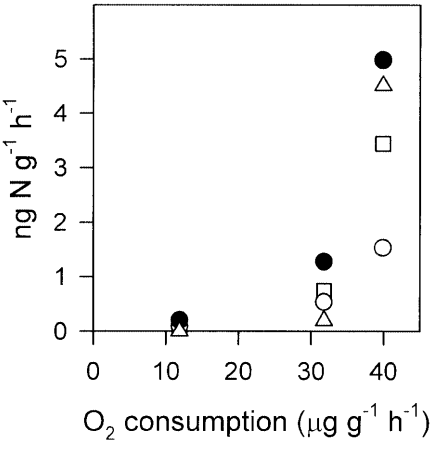

Fig. 6 Average rates of autotrophic (○), heterotrophic (□) and total N_2O production (●) and N_2 production (△) plotted against O_2 consumption rates for the forest soil

Although N_2 production increased more steeply with increasing temperature than heterotrophic N_2O production, up to 40 °C the ratio heterotrophic N_2O/N_2 was never <1, and the overall amount of N_2O-N still exceeded the amount of N_2–N by 10%. Even if the N_2O/N_2 ratio has been observed to decrease with increasing temperature, the present experimental conditions, i.e. a thin layer of soil, large headspace, 60% WFPS, were not favourable for the reduction of N_2O to N_2.

Most of the N_2O production in the forest soil had an autotrophic origin and its rate grew linearly with temperature, rather than exponentially as observed for most biological reactions. This also contrasted with the observations for the arable soil, where autotrophic N_2O production and temperature were related by an exponential relationship of the Arrhenius type. This different relationship with temperature results in a different sensitivity to temperature of the two soils. In fact, while in the forest soil N_2O production increases constantly for each 1 °C increase in temperature, in the arable soil its rate increases faster the higher the temperature. As a result, if a 3 °C increase over the maximum annual temperature (25 °C) is assumed, as forecast by modellers for the present century (IPCC 1995), a 31% relative increase in N_2O production in the arable soil can be calculated, in contrast with only a 10% relative increase in the forest soil.

Despite the low heterotrophic N_2O production, the forest soil showed a significant denitrification activity, yielding mainly N_2. Indeed, the N_2O/N_2 ratio decreased significantly from 0.18 at 25 °C to 0.05 at 40 °C. The Q_{10} for N_2 production, however, was much lower (2.5–2.7) than for the arable soil, which suggests that temperature has a less dramatic effect on soil conditions and on the processes which stimulate N_2 production by denitrification in the forest soil, as compared with the arable soil. These results are in accordance with previous findings on the same soils (Castaldi and Smith 1998), which showed that in the forest soil denitrification yielded mainly N_2, while N_2O was derived mostly from

NH_4^+ oxidation and NO_2^- reduction processes other than denitrification.

Soil respiration increased according to an Arrhenius equation in both soils with a Q_{10} of about 2 (Kirshbaum 1995; Winkler et al. 1996), and it was much higher in the forest than in the arable soil. This was particularly evident with increasing temperature. This type of result should not depend on substrate limitation, because in a 24-h incubation only the forest soil samples incubated at 40 °C produced more CO_2–C than the quantity of added glucose-C. However, the microbial biomass measured in the forest soil immediately after field sampling was sixfold that of the biomass measured in the arable soil (Castaldi 1997). A higher biomass and a natural higher C content of the soil (in the absence of C and N additional amendments) might have induced faster microbial growth in the forest soil during the pre-incubation period, which, in turn, might have led to a faster respiration rate and O_2 consumption rate in the 24-h incubation, despite the addition of the same quantity of glucose and N to both soils.

Relationship between N_2O production, CO_2 production and O_2 consumption at different temperatures

In non-saturated soils, both nitrification and denitrification contribute to N_2O production. A reduction in the O_2 concentration in soil stimulates N_2O production by both processes. However, at low O_2 concentrations nitrification is inhibited, whereas denitrification is at optimal conditions (Granli and Bøckman 1994). It has been suggested that, in aerated soils, denitrification occurs in microsites of intense microbial activity associated with fresh organic matter, the so-called "hot spots" (Parkin and Tiedje 1984). In such microsites the high respiration rate lowers the O_2 concentration. If the rate of O_2 consumption is faster than the rate of O_2 diffusion into the centre of the microsite, denitrification can take place, even if the surrounding environment is "aerobic".

The results show that in both soils the temperature-induced increase in respiration activity was significantly correlated to N_2O production ($P<0.05$). In the forest soil, the increased rates of respiration and O_2 consumption stimulated N_2O production and denitrification. However, the relative increase in the rate of N_2O production slowed down when the concentration of O_2 decreased to $<15\%$ (v/v). This can be explained by the fact that nitrification was the main source of N_2O. In contrast, denitrification and the N_2 production rate rose exponentially with O_2 consumption. This was in accordance with the results of Smith (1980) and Parkin and Tiedje (1984). Nevertheless, only at high rates of O_2 consumption (58 μg O_2 g^{-1} h^{-1}), which occurred at about 34 °C, did the production of N_2 outweigh that of total N_2O-N. Consequently, for the range of temperatures that might be recorded at the forest site during the year, nitrification was the dominant source of N_2O and the ratio N_2O/N_2 was >1.

In the arable soil, although a statistically significant relationship was not obtained, O_2 consumption seemed to stimulate both autotrophic and heterotrophic N_2O production. The relative contribution of nitrification decreased significantly only when the O_2 consumption rate rose above 30 μg O_2 g^{-1} h^{-1} (at about 25 °C). Above this value, heterotrophic N_2O production became predominant and outweighed N_2 production at all temperatures.

In conclusion, in the two soils the dominant source of N_2O was different and seemed to respond differently to temperature changes. In the forest soil the effect of temperature on N_2O production was less dramatic than in the arable soil, as nitrification was the main source of N_2O. A stronger effect of temperature increase on N_2O production was observed in the arable soil, apparently as a result of an incremental contribution of denitrification to the overall N_2O flux. This seemed to depend on the progressively increasing effect of temperature on respiration and the O_2 consumption rate, which enhanced favourable conditions for denitrification.

Even if it is not correct to extrapolate results to field level just on the basis of model laboratory experiments, the effect of temperature on N_2O production, evidenced in this experiment, could be reasonably expected to be more pronounced in the field, where the soil structure contributes to a slowing down of the supply of O_2 to microorganisms. This should be particularly true for the arable soil where N_2O was derived mainly from denitrification. Moreover, for most of the year, such soil would not be limited in C and N substrates due to agricultural fertilization practices.

Acknowledgement This work was supported by an EC fellowship to S Castaldi.

References

Best EK (1976) An automated method for determining nitrate-N in soil extracts. Queensl J Agric Anim Sci 33:161–165

Castaldi S (1997) Microbial processes contributing to N_2O production in two sandy Scottish soils. PhD thesis. Edinburgh University, Edinburgh

Castaldi S, Smith KA (1998) The effect of different N substrates on biological N_2O production from forest and agricultural light textured soils. Plant Soil 199:229–238

Crooke WM, Simpson WE (1971) Determination of ammonium in Kjeldahl digest of crops by an automated procedure. J Sci Food Agric 22:9–10

Davidson EA, Swank WT, Perry TO (1986) Distinguishing between nitrification and denitrification as a source of gaseous nitrogen production in soil. Appl Environ Microbiol 52:1280–1286

Dorland S, Beauchamp EG (1991) Denitrification and ammonification at low soil temperatures. Can J Soil Sci 71:293–303

Granli T, Bíckman OC (1994) Nitrous oxide from agriculture. Norw J Agric Sci [Suppl] 12:1–128

Hynes RK, Knowles R (1978) Inhibition by acetylene of ammonia oxidation in *Nitrosomonas europea*. FEMS Microbiol Lett 4:319–321

IPCC (1990) International Panel on Climate Change: climate change assessment. Cambridge University Press, Cambridge
IPCC (1995) Climate change 1995: the science of climate change. Cambridge University Press, Cambridge
Kirschbaum MUF (1995) The temperature dependence of soil organic matter decomposition and the effect of global warming on soil organic matter. Soil Biol Biochem 27:753–760
Mahli SS, McGill WB, Nyborg M (1990) Nitrate losses in soils: effect of temperature, moisture and substrate concentration. Soil Biol Biochem 22:733–737
Matson PA, Vitousek PM, Schimel DS (1989) Regional extrapolation of trace gas flux based on soils and ecosystems. In: Andreae MO, Schimel DS (eds) Exchange of trace gases between terrestrial ecosystems and the atmosphere. Wiley, Chichester, pp 97–108
Parkin TB, Tiedje JM (1984) Application of a soil core method to investigate the effect of oxygen concentration on denitrification. Soil Biol Biochem 16:331–334
Peterjohn WT, Melillo JM, Bowles FP, Steudler PA (1993) Soil warming and trace gas fluxes: experimental design and preliminary flux results. Oecologia 93:18–24
Powlson DS, Saffigna PG, Kragt-Cottaar M (1988) Denitrification at sub-optimal temperatures in soils from different climatic zones. Soil Biol Biochem 20:719–723
Smith KA (1980) A model of the extent of the anaerobic zones in aggregated soils, and its potential application to estimates of denitrification. J Soil Sci 31:263–277
Smith KA (1997) The potential for feedback effects induced by global warming on emissions of nitrous oxide by soil. Global Change Biol 3:327–338
Winkler JP, Cherry RS, Schlesinger WH (1996) The Q_{10} relationship of microbial respiration in a temperate forest soil. Soil Biol Biochem 28:1067–1072
Yoshinary T, Knowles R (1976) Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochem Biophys Res Commun 69:705–710