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REGULAR ARTICLE

Effects of nitrate concentration on the denitrification potential of a calcic cambisol and its fractions of N_2, N_2O and NO

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Abstract
Background and aims The direct measurement of denitrification dynamics and its product fractions is important for parameterizing process-oriented model(s) for nitrogen cycling in various soils. The aims of this study are to a) directly measure the denitrification potential and the fractions of nitrogenous gases as products of the process in laboratory, b) investigate the effects of the nitrate (NO_3^-) concentration on emissions of denitrification gases, and c) test the hypothesis that denitrification can be a major pathway of nitrous oxide (N_2O) and nitric oxide (NO) production in calcic cambisols under conditions of simultaneously sufficient supplies of carbon and nitrogen substrates and anaerobiosis as to be found to occur commonly in agricultural lands.
Methods Using the helium atmosphere (with or without oxygen) gas-flow-soil-core technique in laboratory, we directly measured the denitrification potential of a silt clay calcic cambisol and the production of nitrogen gas (N_2), N_2O and NO during denitrification under the conditions of seven levels of NO_3^- concentrations (ranging from 10 to 250 mg N kg^{-1} dry soil) and an almost constant initial dissolved organic carbon concentration (300 mg C kg^{-1} dry soil).
Results Almost all the soil NO_3^- was consumed during anaerobic incubation, with 80–88 % of the consumed NO_3^- recovered by measuring nitrogenous gases. The results showed that the increases in initial NO_3^- concentrations significantly enhanced the denitrification potential and the emissions of N_2 and N_2O as products of this process. Despite the wide range of initial NO_3^- concentrations, the ratios of N_2, N_2O and NO products to denitrification potential showed much narrower ranges of 51–78 % for N_2, 14–36 % for N_2O and 5–22 % for NO.
Conclusions These results well support the above hypothesis and provide some parameters for simulating effects of variable soil NO_3^- concentrations on denitrification process as needed for biogeochemical models.

Keywords Nitrogen gas (N_2) · Nitrous oxide (N_2O) · Nitric oxide (NO) · Gas flow soil core technique · Denitrification · Nitrate (NO_3^-)

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Introduction

Denitrification, which is the microbial reduction of nitrate (NO_3^-) or nitrite via nitric oxide (NO) and

nitrous oxide (N_2O) to nitrogen gas (N_2) (Firestone and Davidson 1989; Ye et al. 1994), plays an important role in the global nitrogen cycle because it removes reactive nitrogen from terrestrial and aquatic ecosystems (e.g., Davidson and Seitzinger 2006; Groffman et al. 2006; Seitzinger et al. 2006). In the absence of denitrification, biological or industrial N_2 fixation would eventually deplete the atmospheric N_2, and the biosphere would be inundated with nitrate (Robertson and Groffman 2007). Moreover, N_2O and NO are primary and secondary greenhouse gases, respectively; and they are also important components influencing the atmospheric chemistry (IPCC 2007). Thus emissions of N_2O and NO from upland soils have been intensively investigated (e.g., Bouwman et al. 2002; Stehfest and Bouwman 2006).

Despite the ecological importance of denitrification with N_2 as end product, little information regarding N_2 emissions from upland soils has been obtained by direct measurements. This is mainly due to methodological constraints that do not allow reliable direct measurements against the high background level of 78 % N_2 in the atmosphere (Groffman et al. 2006). So far, indirect measurements of N_2 emissions from upland soils have been widely performed with the acetylene (C_2H_2) inhibition technique (e.g., Ciarlo et al. 2008; Yoshinari et al. 1977) and/or the ^{15}N tracing method (e.g., Mathieu et al. 2006; Ruser et al. 2006). The former technique has been criticized because the denitrification rates can be severely underestimated by even up to a factor of 10 (Bollmann and Conrad 1997), mainly due to a) inhibition of nitrification (Mosier 1980), b) incomplete blockage of the N_2O reductase if the soil NO_3^- concentrations are low (Simarmata et al. 1993), and c) uneven distribution of the inhibitor (Jordan et al. 1998). The latter method is insensitive to measure N_2 emissions, due to a high detection limit of 180 μg N m^{-2} h^{-1} or 43 g N ha^{-1} d^{-1} (Ruser et al. 2006). Recently, the gas-flow-soil-core technique has been increasingly used for direct quantification of N_2 emissions from soils (Butterbach-Bahl et al. 2002; Cárdenas et al. 2003; Dannenmann et al. 2008; De Wever et al. 2002; Scholefield et al. 1997a,b; Wang et al. 2011; Senbayram et al. 2011). Using this method, the soil air is replaced with an artificial N_2-free gas, and then direct measurements of N_2, N_2O and NO emissions are performed. The shortcomings of this technique are mainly associated with the a) long time (20–48 h) needed to firmly establish an N_2-free environment, b) low sensitivity of N_2 detection (>10 μg N m^{-2} h^{-1}), and c) strict gas-tightness requirement of the incubation system (Groffman et al. 2006). To overcome these shortcomings, the modification of hardware and an improvement in operational procedures have been recently achieved for the gas-flow-soil-core technique, which include a) using a new design of incubation vessels, b) employing a new micro gas chromatography (GC) equipped with thermal conductivity detector (TCD) to assure a small internal volume to improve the N_2 signal quality, c) completing the gas replacement within 25 h via alternating negative pressure and pressurized purging, and d) adopting aerobic, low temperature conditions to maintain the carbon and nitrogen substrates during gas replacement (Wang et al. 2011). These developments have resulted in a better sensitivity in the direct measurement of N_2 emissions, with a detection limit of 0.23 μg N_2–N kg^{-1} ds h^{-1} or 8.1 μg N_2–N m^{-2} h^{-1} and a currently unachieved reproducibility of measurements. Moreover, these developments also allow for simultaneous measurements of NO, N_2O and CO_2 emissions with high sensitivities (Wang et al. 2011).

Due to the ecological significance of N_2, N_2O and NO emissions from soils, quantification of their magnitudes at various timescales (e.g., hourly, daily, annual) and spatial scales (e.g., site, regional) are necessary. Until now, N_2 emissions could not be directly measured in the field at any scale. Meanwhile, emissions of N_2O and NO can be directly measured at the plot scale and on different timescales, but cannot be easily observed at the regional scale. Therefore, modeling approaches have to be established to quantify the emissions of these gases at different temporal and spatial scales. For simulating the ecosystem nitrogen cycling, it is necessary to parameterize key nitrogen cycling processes such as denitrification. However, due to the already mentioned methodological problems, we are lacking parameter kinetics and a detailed evaluation of the production of N_2, N_2O and NO for denitrification.

Generally, denitrification in the calcic cambiols as found on the North China plain is regarded as weak and unimportant for the emissions of N_2O and NO and losses of nitrate (e.g., Cai et al. 2002; Ju et al. 2009; Wan et al. 2009), although N_2 emissions from calcareous soils may be high as shown e.g., by Dannenmann et al. (2008). Nevertheless, data from Liu et al. (2012) shows that during the maize season (June–October) the calcic cambisols often contains very high soil moisture contents, with values around or above 65–70 % water-

filled pore space (WFPS) for up to 2–3 weeks. During these periods soils are often weak sources for atmospheric methane (CH_4), indicating that predominantly anaerobic conditions prevail. Therefore, denitrification is likely to be important for N_2O and NO emissions from these soils under conditions of sufficient supplies of carbon and nitrogen substrates and anaerobiosis (Mei et al. 2009, 2011). Moreover, direct measurement of the denitrification potential and its N_2, N_2O and NO fractions for calcic cambisols is very important in terms of model parameterization and following model estimation of denitrification on a regional scale. However, few directly measured data on the denitrification potential and the production of N_2, N_2O and NO during denitrification can be found for this soil type.

In this study, we performed laboratory experiments for a silt clay calcic cambisol using the gas-flow-soil-core technique (Wang et al. 2011). The objectives were to a) directly measure the denitrification potential and the fractions of nitrogenous gases as products of the process, b) investigate the influences of the NO_3^- concentration on denitrification gas emissions, and c) test the hypothesis that denitrification is important for N_2O and NO emissions from calcic cambisols under conditions of sufficient supplies of carbon and nitrogen substrates and anaerobiosis as to be found to occur commonly in agricultural lands.

Materials and methods

Soil

The soil used for this experiment was collected (at a depth of 0–15 cm) from a field (34 °55.51′N, 110 ° 42.59′E, 348 m altitude) cultivated with winter wheat and summer maize in rotation (Shanxi, China). The field site was involved in a study aiming at the quantification of greenhouse gas emissions (Liu et al. 2011, 2012). It exposes to a monsoon climate of the southern temperate zone, with annual mean air temperature and precipitation of 14.8°C and 562 mm, respectively, during 2000–2008 (National Climatic Data Center, http://www.ncdc.noaa.gov/oa/ncdc.html). The field site is dominated by a cinnamon soil (classified by the National Soil Survey Office 1998) or calcic cambisol (classified by WRB 2006), which contains 31.8±0.9 % clay, 38.9±1.8 % silt, 29.3±2.5 % sand, 1.13±0.06 % organic carbon, 0.11±0.005 % total nitrogen and has a pH (H_2O) of 8.7±0.05, and a bulk density of 1.17±0.04 gcm^{-3} (Liu et al. 2011). Bulk soil samples were air dried to a gravimetric moisture content of 18 % (ca. 32 % WFPS), sieved with a 2 mm sieve, mixed and stored at 4°C.

Experimental design

For determining the effect of variation in soil NO_3^- on denitrification rates and denitrification products, NO_3^- was added to soil samples to obtain initial NO_3^- concentrations of approximately 10, 30, 50, 80, 100, 180 and 250 mg N kg^{-1} dry soil (hereafter referred to as 10 N, 30 N, 50 N, 80 N, 100 N, 180 N and 250 N, respectively). These initial NO_3^- concentrations in soils are equivalent to 4.2–107 kg N ha^{-1}. The 10 N treatment, with a background NO_3^- concentration of 12.9 mg N kg^{-1} ds in the soil samples, was free from NO_3^- addition and used as the control (Table 1). In all treatments the initial dissolved organic carbon (DOC) concentrations were set at 300 mg C kg^{-1} dry soil (ds). Hereafter the sum of background NO_3^- (or DOC) concentration in soil samples and the NO_3^- (or DOC) added prior to each treatment is referred to as the initial NO_3^- (or DOC) concentration.

For all treatments the same soil was used. But due to the length of experiments, we re-started the experiment if a new NO_3^- level was investigated (i.e., each treatment was carried out with the same soil, which was not used previously for other treatments, but was taken from the same pool of air dried soil samples stored in our laboratory). For each treatment, two weeks before the start of measurements, the air dried soils were wetted with deionized water to approximately 45 % WFPS and pre-incubated at 4°C. This means, as already said before, that we used a new freshly pre-incubated soil for every NO_3^- amendment treatment. The soil moisture content was held stable during pre-incubation, by adding certain amounts of water following daily weighing. For each treatment, the pre-incubated soil was repacked into 12 cores (bulk density: 1.07 g cm^{-3}) using standard stainless steel rings (diameter 5.6 cm, height 4 cm). Four cores were placed into one of the three incubation vessels. Sub-samples taken from the pre-incubated soil were extracted for NO_3^- and DOC assay in order to determine the amount of NO_3^- and glucose additions. The measured concentrations of NO_3^- and DOC varied within a range of 5–19 mg N kg^{-1} ds and 30–50 mg C kg^{-1}

Table 1 Cumulative emissions of N_t (N_2+N_2O+NO) and carbon dioxide (CO_2), and changes in ammonia (NH_4^+), nitrate (NO_3^-), dissolved organic carbon (DOC), soil microbial biomass nitrogen (SMBN) and carbon (SMBC), and the recovery rate of carbon and nitrogen during the entire incubation period under different initial nitrate (NO_3^-) concentrations

		10 N	30 N	50 N	80 N	100 N	180 N	250 N
N_t		10.3±0.6	25.2±0.3	43.4±0.4	63.0±1.4	89.8±0.9	163.9±1.6	208.9±4.5
CO_2		68.1±5.2	58.7±1.0	79.3±8.5	59.3±2.8	96.8±6.7	152.4±3.7	161.1±1.1
NO_3^-	c_0	12.9±0.6	11.3±0.2	4.9±0.5	6.9±0.5	5.6±0.3	19.2±0.5	10.1±0.5
	A_n	0	19.6	44.5	72.8	97.4	168.5	244.0
	c_1	0.1±0.01	0.3±0.05	0.2±0.01	0.1±0.01	0.2±0.01	1.5±0.2	0.4±0.01
	Δc	12.8±0.6	30.5±0.2	49.3±0.7	79.7±0.7	102.8±0.5	186.3±0.9	253.7±2.0
NH_4^+	c_0	0.02±0.02	0	0.2±0.02	0.3±0.04	0.6±0.1	1.3±0.2	0.3±0.2
	c_1	5.2±0.3	2.7±0.2	9.0±0.9	3.2±0.7	6.4±0.4	12.5±0.3	21.0±0.4
	Δc	−5.2±0.3	−2.7±0.2	−8.8±0.9	−2.9±0.4	−5.7±0.4	−11.2±0.3	−20.7±0.4
SMBN	c_0	31.4±1.4	59.3±1.3	53.2±1.5	39.0±1.0	48.1±1.7	38.1±1.6	54.3±2.9
	c_1	26.1±0.9	58.1±1.2	53.3±2.5	38.1±0.7	45.2±3.0	22.7±2.7	48.7±1.7
	Δc	5.4±1.7	1.3±1.8	−0.1±2.9	0.9±1.2	2.9±3.5	15.5±3.1	5.6±3.3
DOC	C_0	28.6±2.4	36.7±1.9	46.2±2.7	33.2±2.5	41.9±3.7	51.2±1.6	44.3±3.1
	A_c	274.3	249.7	250.5	203.2	250.8	270.2	270.1
	c_1	131.3±1.0	101.9±4.0	128.9±6.9	110.6±7.9	110.5±4.7	84.0±2.3	47.2±0.2
	Δc	171.6±3.0	184.5±4.5	167.8±7.7	125.9±8.7	182.2±6.0	237.4±3.0	267.3±3.8
SMBC	c_0	183.7±5.6	266.2±8.9	262.3±80.3	185.4±55.8	256.3±13.0	185.0±5.5	194.2±10.0
	c_1	196.5±6.0	282.3±12.6	296.0±84.9	234.6±48.1	290.4±37.5	164.0±2.6	217.8±3.8
	Δc	−12.7±8.2	−16.1±15.4	−33.6±116.8	−49.3±73.4	−34.1±39.7	21.0±6.1	−23.6±10.7
N-RR_n (%) [a]		80.8±6.0	82.6±1.1	88.1±1.4	79.0±1.8	87.3±0.9	88.2±1.0	82.3±1.9
							[84.4±0.9]	[53.9±0.7]
N-RR_f (%) [b]		79.6±11.9	86.8±5.6	107.6±8.3	81.2±2.4	89.8±3.3	86.1±1.7	87.5±2.4
							[82.5±1.6]	[57.3±1.1]
C-RR_d (%) [c]		39.7±3.1	31.8±0.9	47.3±5.5	53.4±4.0	53.1±4.1	64.2±1.8	60.3±1.0
							[53.4±1.6]	[40.3±0.7]
C-RR_f (%) [d]		42.9±4.0	34.9±3.4	59.1±5.5	87.7±4.0	65.1±4.1	59.0±2.1	66.1±3.1
							[49.3±1.9]	[44.2±2.1]

10N, 30 N, 50 N, 80 N, 100 N, 180 N and 250 N denote the initial NO_3^- treatments of approximately 10, 30, 50, 80, 100, 180 and 250 mg N kg^{-1} dry soil (ds), respectively. c_0 represents the concentrations at the end of pre-incubation. c_1 denotes the concentrations at the end of incubation. A_n and A_c are the added nitrogen in form of KNO_3 and carbon in the form of glucose prior to incubation, respectively. Δc denotes the changes of the concentrations during incubation. The emissions and concentrations were expressed in unit of mg N or C kg^{-1} ds. Means ± standard error of at least three independent replicates are given.

[a] N-RR_n is the nitrogen recovery rate, which is the ratio of N_t (the sum of cumulative N_2, N_2O and NO emissions) to the change in the soil NO_3^- pool. The data outside the brackets are the recovery rates over the entire incubation period, and those inside are the recovery rates within the period of 0–240 h.

[b] N-RR_f is the nitrogen recovery rate, which is the ratio of N_t to the sum of the changes in the soil pool of NO_3^-, NH_4^+ and SMBN. The definition of the data inside and outside the brackets is the same as in b.

[c] C-RR_d is the carbon recovery rate, which is the cumulative CO_2 emissions expressed as a percentage of the change in the soil DOC pool. The definition of the data inside and outside the brackets is the same as in b.

[d] C-RR_f is the carbon recovery rate, which is the cumulative CO_2 emissions expressed as a percentage of the sum of the changes in the soil DOC and SMBC pool. The definition of the data inside and outside the brackets is the same as in b.

ds, respectively (shown as C_0 in Table 1). Accordingly, 5 mL of solution with appropriate KNO_3 and glucose concentrations was sprinkled onto the surface of each soil core to obtain the desired initial NO_3^-

concentrations (approximately 10–250 mg N kg^{-1} ds) and a fixed initial DOC concentration (approximately 300 mg C kg^{-1} ds) (Table 1). Addition of the solution increased the soil moisture content from 45 % to 55 % WFPS.

Immediately following the solution additions, the vessels were sealed gas tight (see Wang et al. 2011) and submerged into a water bath for temperature control as well as reducing possible gas diffusion between atmosphere and vessels containing the soil cores. Then the soil atmosphere was purged at 2°C, using an N_2-free atmosphere of 20 % oxygen (O_2) in helium (He). During the aerobic condition at low temperature, the consumptions of the added carbon and nitrogen substrates is negligible as was shown by Wang et al. (2011). Hereafter we refer to the period replacing the soil atmosphere with an N_2-free gas at low temperature the "gas exchange phase". The gas exchange phase was set to last for 30 h, during which the replacement of soil atmosphere was carried out by alternating negative pressure (by evacuation for 2 min using an vacuum pump to approximately 30 kPa) followed by pressurized purging with N_2-free atmosphere for 2 min at a flow rate of 200 mL min^{-1} (Wang et al. 2011). This phase was followed by gas emission measurements carried out for approximately 15 h under the aerobic condition at 2°C. Then an anaerobic condition was established by flushing the vessel headspace with pure He. After 48 h of anaerobic incubation at 2°C, the temperature was increased. It took 40 min to achieve the target temperature of 25°C (Fig. 1). Incubation conditions of 25°C and 55 % WFPS were chosen since these conditions are typical in soils of the field during the maize growing season (Liu et al. 2012). In our experiment anaerobic

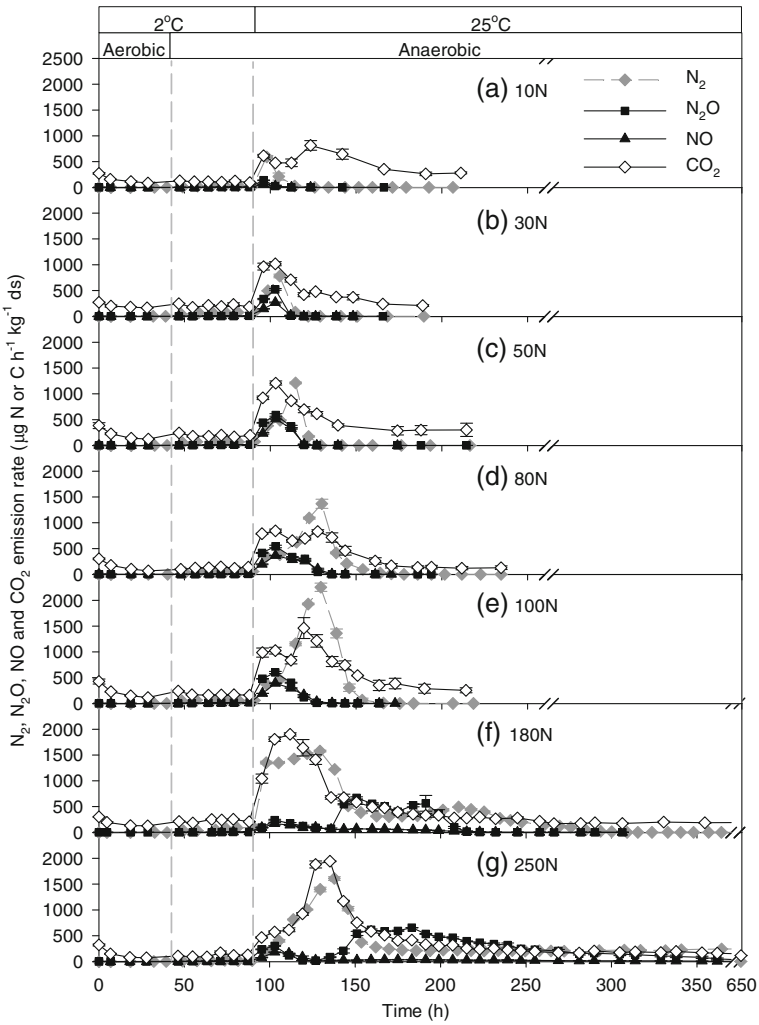

Fig. 1 Dynamics and magnitudes of nitrogen gas (N_2), nitrous oxide (N_2O), nitric oxide (NO) and carbon dioxide (CO_2) emissions for the different soil nitrate (NO_3^-) addition treatments (10–250 mg N kg^{-1} ds). Definitions of the treatment codes are referred to in the text and Table 1. Time zero represents the beginning of the incubation, i.e., following the closure of the vessels and the equilibration of the water bath temperature to 2°C. T=0 directly follows the addition of NO_3^- and setting of soil dissolved organic carbon (DOC) concentrations to 300 mg C kg^{-1} ds by the addition of the respective amounts of glucose. The means of three replicate experiments ± standard error are shown

incubations were provided by replacing the soil atmosphere with pure He. The O_2 concentrations were monitored with an electron capture detector (ECD) and TCD (see below). Both detectors demonstrated that no O_2 was available under the chosen incubation conditions. In the gas exchange phase, N_2 could not be measured, whereas the emissions of other gases (N_2O, NO and CO_2) were measured once every 5–10 h. Following complete gas exchange, the N_2, N_2O, NO and CO_2 emissions were simultaneously measured once every 8 h. Gas measurements for a treatment were stopped when the emissions of nitrogenous gases declined to around the detection limits of the employed gas-flow-soil-core system.

Gas sampling and analysis

The semi-dynamic chamber method described by Wang et al. (2011) was applied to determine the emissions of the individual gases (N_2O, NO, CO_2 and N_2). To take an air sample for detecting N_2O, NO and CO_2 concentrations, a gas containing 20 μL N_2 L^{-1} (ppmv) in He was allowed to continuously flush the vessel headspace at 20 mL min^{-1} for 4 min. The out-flowing air during the last 3 min was collected with a 60-mL syringe. Then we halted the headspace flushing for 11 min before initiating the collection of the next sample. This procedure was repeated 5 times, i.e., the vessel headspace air for simultaneous detection of N_2O, NO and CO_2 was sampled at an interval of 15 min over a 1-hour period. Out of each 60 mL gas sample, 20 mL was used to analyze the N_2O and CO_2 concentrations using a GC (Agilent GC 6820, Shanghai, China) equipped with an ECD and a flame ionization detector (FID) fitted with a convertor to reduce CO_2 into methane. Pure N_2 (99.999 %) was used as the carrier gas for the analysis of both components. For the N_2O analysis we applied the DN-CO_2 method, in which a buffering gas of 10 % CO_2 in pure N_2 flowing through the ECD cell at approximately 2 mL min^{-1} was used for N_2O detection. Details regarding the GC configurations for analysis of the two gases can be found in Zheng et al. (2008) and Wang et al. (2010). The remaining 40 mL of the gas sample was diluted to a volume of 2 L by injecting it into a gas-tight Tedlar membrane bag (Delin Gas Packing Co. Ltd., Dalian, China) pre-filled with pure N_2 (99.999 %). Then the NO concentrations were measured with a chemiluminescent analyzer (42i NO–NO_2–NO_X, Thermo Environmental Instruments Inc., USA). To detect the N_2 emission, we used a sampling interval of 45 min over a 3 h period. The procedures of the detector calibration with a standard gas and flushing all tubes prohibited a shorter interval of measuring N_2 concentration in the vessel headspace. The N_2 concentrations were automatically detected by online analysis following injection of the out-flowing air (immediately after flushing the headspace of the vessel with 20 ppmv N_2 in He for 3.8 min at a flow rate of 20 mL min^{-1}) into a micro GC equipped with a TCD (Agilent micro GC 3000, USA). Then we halted the headspace flushing for 41 min before initiating the collection of a new sample. This procedure was repeated 5 times. The measured N_2 concentrations were corrected by subtracting the inherent N_2 leakage rate of the system, which was 0.4 ppmv h^{-1} on average. Details regarding the GC configurations used for the N_2 analysis can be found in Wang et al. (2011).

All instruments were regularly calibrated with standard gases (AP BEIFEN Gases Industry Co. Ltd., Beijing, China). The standard gas concentrations of N_2 and CO_2 were 20 ppmv (in He) and 354 ppmv (in N_2), respectively. Over the wide ranges of N_2 (20–2000 ppmv) and CO_2 (300–3000 ppmv) concentrations in the air samples, the detector always shows significant linear response, with determination coefficient of the linear regressions (R^2) greater than 0.999. A series of N_2O standard gases (with 0.352, 5, 20, and 200 ppmv N_2O in N_2) was employed to obtain calibration curves for routine use.

Calculation of emissions

Since the headspace air was diluted by the flushing process during sampling, as described above, the measured concentrations had to be corrected before they were used in the calculation of emissions. The correction was based on Eqs. 1–2 (see Wang et al. 2011 for details).

$$C_i^* = C_i^m - \sum_1^i e^{bi} \cdot \left[\mathrm{e}^{\left(\frac{-v_{in}}{V_{head}} \cdot t \right)} - 1 \right] \quad \text{when } C_i^m > C_{in} \tag{1}$$

$$bi = \ln\left(\left| C_i^m - C_{in} \right| \right) + \frac{v_{in}}{V_{head}} \cdot t \tag{2}$$

where C_i^* is the corrected concentration of a gas (ppmv); t is the sampling time during headspace

flushing (min); i is the series number of measurements for determining an emission (i=1,2, …, 5); C^m is the measured concentration of the gas (ppmv); C_{in} is the inlet gas concentration during sampling (ppmv); V_{head} is the headspace volume (mL); v_{in} is the inlet gas flow rate during sampling (mL min^{-1}); and bi is a parameter specific for each concentration measurement.

The emission of a gas was determined using Eq. 3 (Wang et al. 2011).

$$F = \frac{V_{head} \cdot \left(\Delta C_i^* - \Delta C_L\right) \cdot M}{M_{ds} \cdot MV} \cdot \frac{273}{273 + T} \quad (3)$$

where F is the emission of a gas (μg N or C h^{-1} kg^{-1} ds); ΔC_i^* is the change rate in the corrected concentration of a gas (ppmv h^{-1}); ΔC_L is the inherent leakage rate of the system for the gas (N_2: 0.4 ppmv $N_2 h^{-1}$; all other gases: negligible); M is the weight of pure nitrogen or carbon per mole in N_2, N_2O, NO or CO_2 (28, 28, 14 and 12 g mol^{-1}, respectively); M_{ds} is the dry weight of the incubated soil (g) in an vessel; MV is the molar volume of the gas at 273 K and 1,013 hPa (L mol^{-1}); and T is the incubation temperature (°C). The detection limits of our gas-flow-soil-core system were 0.23, 0.02 and 0.08 μg N h^{-1} kg^{-1} ds (or 8.1, 0.6 and 2.7 μg N m^{-2} h^{-1} for the soil cores height of 4 cm) for N_2, N_2O and NO, respectively, and 1.9 μg C h^{-1} kg^{-1} ds (or 67.2 μg C m^{-2} h^{-1}) for CO_2.

Soil analysis

At the beginning and end of incubation for a treatment, we measured the concentrations of NO_3^-, ammonium (NH_4^+), DOC, and microbial biomass carbon and nitrogen (hereafter referred to as SMBC and SMBN, respectively) in the incubated soils. For analysis of the inorganic nitrogen content, 20 g of soil was extracted for 1 h, using 100 mL of deionized water for NO_3^- or 100 mL of a 2 M potassium chloride (KCL) solution for NH_4^+ (Keeney and Nelson 1982). The NH_4^+ concentrations were determined colorimetrically using an ultraviolet spectrophotometer (UNICO, UV-2802, Shanghai, China), while the NO_3^- contents were determined by ion chromatography (Metrohm 790 IC, Switzerland). The DOC was extracted by shaking 20 g of the soil sample for 1 h with 100 mL of deionized water. The extracts were centrifuged at 6000 rpm for 15 min and decanted, and the supernatant was then filtered through a 0.45 μm polyethersulfone membrane filter (Membran, Germany) before analysis with a C/N analyzer (multi NC 3000, Analytik, Jena, Germany). The SMBC or SMBN content was determined from the difference in the concentrations of DOC or dissolved nitrogen extracted from the fumigated and non-fumigated soils (7 g was extracted using 35 mL of 0.5 M potassium sulfate) (Sparling and West 1988).

Statistics

The software package SPSS Statistics Client 19.0 (SPSS, Beijing, China) was used for data analysis (ANOVA and GLM regressions). Graphical outputs were obtained from Origin 8.0 (Origin Lab Ltd., Guangzhou, China).

Results

Emission dynamics of nitrogenous gases and CO_2

The emission dynamics of nitrogenous gases and CO_2, as affected by the different levels of initial NO_3^- concentrations, are displayed in Fig. 1, while the times of the emission peaks of the nitrogenous gases are listed in Table 2.

During aerobic incubation at 2°C, the N_2O and NO emissions were generally close to their detection limits, whereas the N_2 emissions were slightly higher than its detection limit. The CO_2 emissions were initially at 400–500 μg C h^{-1} kg^{-1} ds and decreased to approximately 120 μg C h^{-1} kg^{-1} ds.

The N_2 emissions during the anaerobic incubation at 2°C were in the range of 20–100 μg N h^{-1} kg^{-1} ds, while N_2O emissions were at the same magnitude as NO emissions, but by a factor of 3–10 lower than N_2 emissions. The CO_2 emissions were 100–260 (mean: 160) μg C h^{-1} kg^{-1} ds. The emissions of all nitrogenous gases during the conditions of anaerobiosis and marginally low temperature were significantly increased by 1–2 orders of magnitude compared to the aerobic conditions ($p<0.01$), while the soil respiration, as indicated by CO_2, showed no significant difference.

After the target incubation temperature of 25°C was achieved (>95 h), the emissions of nitrogenous gases immediately increased, but the time to reach the maximum varied among the treatments. Under the 10 N condition, the emissions of all of the nitrogenous gases peaked at the first measurement that was performed

Table 2 Magnitude and timing of peak nitrogen gas (N_2), nitrous oxide (N_2O), nitric oxide (NO) emissions for the different nitrate addition treatments (10–250 mg N kg^{-1} ds)

Treatment[a]		Peak time (h)[b]			Peak emission (μg N h^{-1} kg^{-1} ds)[c]		
		N_2	N_2O	NO	N_2	N_2O	NO
10 N		98/3	96/1	96/1	590±34	140±6	63±6
30 N		105/10	103/8	103/8	782±61	523±26	269±16
50 N		115/20	103/8	103/8	1211±11	591±17	516±5
80 N		130/35	103/8	103/8	1368±119	543±18	366±12
100 N		130/35	103/8	103/8	2252±83	602±26	393±18
180 N	Peak 1	130/35	103/8	103/8	1575±18	240±14	170±23
	Peak 2	210/115	150/55	150/55	490±11	670±28	72±2
250 N	Peak 1	138/43	103/8	103/8	1608±37	300±4	185±2
	Peak 2	364/269	183/88	183/88	243±7	656±3	38±2

[a] Definitions of the treatment codes refer to the footnotes of Table 1.

[b] The number before the slash (/) indicates the time of the whole incubation onward, and that after the slash is the time from the change in the incubation temperature from 2°C to 25°C onwards.

[c] Mean values ± standard error of three independent measurements are shown.

within 3 h after the target incubation temperature was archived, and then quickly declined over 28 h (i.e., 126 h after the incubation began) to 1–2 μg N h^{-1} kg^{-1} ds of N_2 and to near the detection limits of N_2O and NO. For 30 N, 50 N, 80 N and 100 N, the N_2O and NO emissions in the first few hours were higher compared to the N_2 emissions. Maximum N_2O and NO emissions were observed by the second measurement (i.e., approximately 103 h after the start of the incubation or 8 h following the temperature increase), which were followed by the N_2 peak. The lag time between the appearance of the N_2O and N_2 peaks was approximately 2 h for 30 N, 12 h for 50 N, and 27 h for 80 N and 100 N. The dynamic emission patterns of all nitrogenous gases observed under 180 N and 250 N conditions showed two peaks. This differed markedly from the patterns of the other treatments that showed single peak of each nitrogenous gas. The N_2O and NO peaks were simultaneously observed, with their first peaks appeared at 103 h, for all treatments excluding 10 N. The second N_2O peak was higher than the first one by 1–4 folds ($p<0.001$) and appeared at 150 h (180 N) or 183 h (250 N) after the incubation started. Meanwhile, the second NO emission was much smaller than the first one. Each N_2 peak followed those of N_2O and NO. The first N_2 peak was much higher than the second one by 2–6 folds ($p<0.001$). Subsequently, N_2 was the sole end product of denitrification.

During the anaerobic incubation at 25°C, as Fig. 1 shows, the emissions of nitrogenous gases peak in the following sequence: $NO \leq N_2O < N_2$. The time (y, in h) required for the first appearance of an N_2 emission peak to occur after raising the temperature increased linearly with the logarithm of the initial NO_3^- concentration (x, in mg N kg^{-1} ds), which could be fitted with $y = 13.9 \ln(x) - 33.0$ ($R^2 = 0.92$, $p<0.001$). The lag time (y, in h) of the first/single N_2 peak, relative to appearance of the first/single N_2O peak, was approximately 2–35 h, also showing a trend of a linear increase with the logarithm of initial NO_3^- concentrations, with $y = 12.0 \ln(x) - 32.2$ ($R^2 = 0.87$, $p<0.05$). As Figs. 2a–c illustrate, the maximum emissions of the nitrogenous gases were significantly affected by the initial soil NO_3^- concentrations, with the relationship for either N_2 or N_2O being very well described by Michaelis-Menten functions ($p<0.01$) and for NO by a log normal curve ($p<0.05$).

With regard to the CO_2 emissions after the temperature increase, the first and in some cases the single peak appeared synchronically with the first/single N_2O peak, while the second peak appeared at the same time as or slightly earlier than the maximum N_2 emission. Towards the end of the incubations, the CO_2

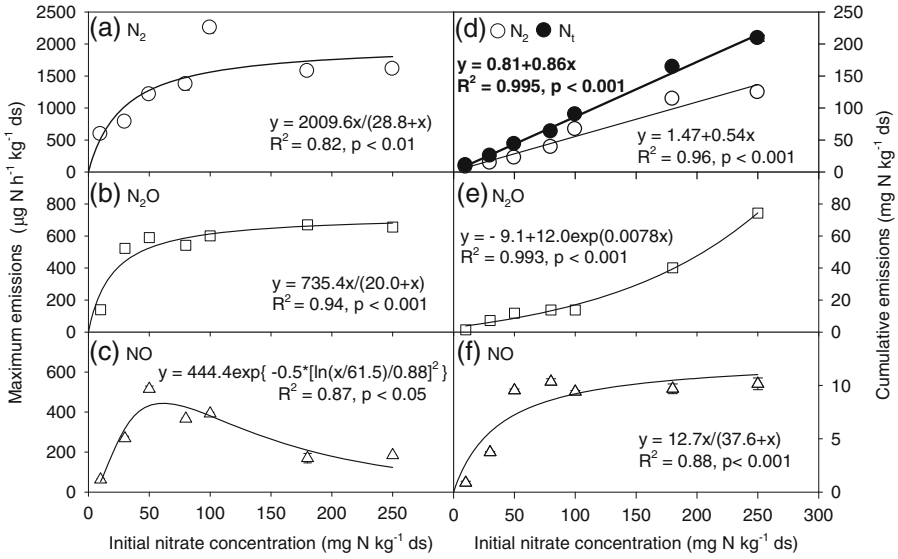

Fig. 2 The dependences of maximum nitrogen gas (N_2), nitrous oxide (N_2O), nitric oxide (NO) emissions (**a**–**c**) and their cumulative emissions over the entire incubation period (**d**–**f**) on initial soil nitrate (NO_3^-) concentrations (10–250 mg N kg^{-1} ds). The data displayed here are the means of three replicate experiments ± standard error

emissions declined and remained constant at approximately 100–300 mg C h^{-1} kg^{-1} ds.

Cumulative emissions of nitrogenous gases and CO_2

The cumulative emissions of the N_2, N_2O, NO and N_2+N_2O+NO (hereafter referred to as N_t) from all the treatments ranged from 8.0–123.4, 1.4–74.4, 0.9–10.1 and 10.3–208.9 mg N kg^{-1} ds, respectively (Table 1 and Fig. 2). As Fig. 2d illustrates, the cumulative N_t and N_2 emissions were linearly and positively correlated with the initial soil NO_3^- concentrations (N_t: R^2=0.995, p< 0.001; N_2: R^2=0.96, p<0.001). Such a relationship exhibited the first order kinetics, which indicated the phase of Michaelis-Menten kinetics occurring at low substrate concentrations. The cumulative emissions of NO obtained in all treatments very well fitted the Michaelis-Menten kinetics (Fig. 2f), whereas the cumulative N_2O emissions depended exponentially upon the initial NO_3^- concentrations (Fig. 2e). With respect to the total cumulative nitrogenous gas emissions due to denitrification, the fractions of the N_2, N_2O and NO emissions to the denitrification potential were in the range of 51–78 %, 14–36 % and 5–22 %, respectively (Fig. 3). These fractions well supported the hypothesis that denitrification is significant for N_2O and NO emissions from calcic cambisols under conditions of sufficient supplies of carbon and nitrogen substrates and anaerobiosis. The cumulative CO_2 emissions during the entire incubation period varied from 68.1 to 161.2 mg C kg^{-1} ds among the treatments (Table 1), with a positive relationship with the initial NO_3^- concentrations (R^2=0.88, p<0.01).

Ratios of cumulative gas emissions

Regarding the cumulative emissions during anaerobic incubation at 25°C, the N_2:N_2O molar ratios measured in all treatments were 1.7–5.3 (Table 3), showing no obvious simple relationship against initial NO_3^- concentrations. The NO:N_2O molar ratios for 180 N and 250 N were less than 0.5 while those for the other treatments were 1.0–1.5 (Table 3), indicating the influence of initial nitrogen substrate concentration to

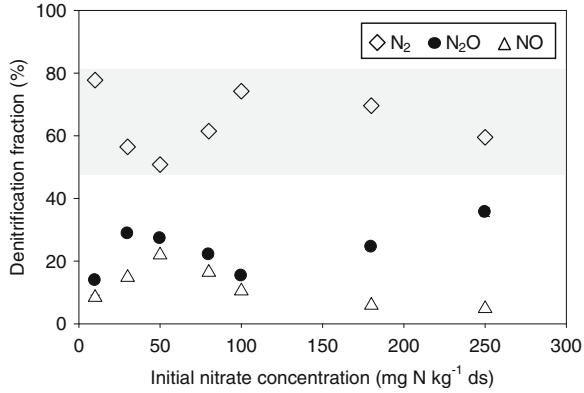

Fig. 3 Effects of different starting soil nitrate (NO_3^-) concentration on the denitrification fraction during the entire incubation period. The means of three replicate experiments ± standard error are given

some extent. The CO_2:N_t molar ratios for 80 N, 100 N, 180 N and 250 N were in a range of 0.9–1.1, and those for the other treatments with lower NO_3^- application rate were 1.9–7.9 (Table 3). The CO_2:N_t molar ratios (y) of all treatments were negatively dependent upon the initial NO_3^- concentrations (x, in mg N kg^{-1} ds). This dependence could be described with a power function as $y=32.8\times^{-0.71}$ ($R^2=0.88$, $p<0.001$).

Nitrogen and carbon balance

In addition to the cumulative N_t and CO_2 emissions, Table 1 provides an overview of the NH_4^+, NO_3^-, SMBN, SMBC and DOC contents at the beginning and end of incubation for the various soil NO_3^- treatments. As can be deduced from Table 1, the soil microbial biomass carbon to nitrogen ratios fell within the range of 4.5–7.5 (mean: 5.5). The biomass carbon and nitrogen of soil biomass, as well as their ratios showed no significant change between the beginning and end of incubation in any of treatments. However, significant changes in the soil NO_3^- contents were observed ($p<0.01$), with the NO_3^- pool being almost completely depleted. A small but significant ($p<0.01$) increase in the soil NH_4^+ concentrations was observed. The observed cumulative N_t emissions equaled 80–88 % (average: 84 %) of the reduction in soil NO_3^-(expressed as N-RR_n values in Table 1) and 80–108 % (average: 88 %) of the total changes in (NH_4^+ + NO_3^-+SMBN) - N (expressed as N-RR_f values in Table 1). Additionally, the observed CO_2–C emissions corresponded to 32–64 % (average: 50 %) of the decrease in soil DOC or 35–88 % (average: 59 %) of the changes in DOC plus SMBC.

Table 3 Molar ratios of cumulative nitrogenous gas and carbon dioxide (CO_2) emissions during anaerobic incubation at 25°C

Treatment[a]	Molar ratios		
	N_2:N_2O	NO:N_2O	CO_2:N_t[b]
10 N	5.3±0.2	1.1±0.1	7.9±1.5
30 N	1.6±0.04	1.0±0.02	2.3±0.01
50 N	1.7±0.1	1.6±0.04	1.9±0.2
80 N	2.7±0.1	1.5±0.04	1.1±0.1
100 N	4.9±0.1	1.4±0.03	1.1±0.1
180 N	2.8±0.1	0.5±0.01	1.0±0.02
250 N	1.7±0.1	0.3±0.01	0.9±0.01

Means ± standard errors for three replicates are given.

[a] Definitions of the treatment codes refer to the footnotes of Table 1.

[b] N_t=N_2+N_2O+NO. CO_2:N_t is calculated using the cumulative emissions in moles of carbon and nitrogen elements.

Discussion

Effect of initial NO_3^- concentrations on peak emissions of denitrification gases

Following induction of anaerobiosis and an increase in incubation temperature from 2°C to 25°C, the first/single peak of N_2 emissions appeared later than the first/single peak of N_2O or NO (Fig. 1 and Table 2). The lag period between the appearance of N_2O and N_2 peaks (2–27 h) was comparable to the 6–12 h observed by Mathieu et al. (2006) or the 19 h lag period reported in the study of Meijide et al. (2010). Dendooven and Anderson (1994) suggested that the lag time between the appearance of the N_2O and N_2 peak resulted from the different lag times for the synthesis of the enzymes of the denitrification chain involved in the production and consumption of N_2O under anaerobic conditions. Another possible explanation for the lag time is the preferential acceptance of electrons from NO_3^- compared with N_2O (Firestone and Tiedje 1979). The latter explanation is well supported by our result that the lag period length was increased linearly with the logarithm of the initial NO_3^- concentration for denitrification ($p<0.05$).

Only a few previous studies, in which the gas-flow-soil-core technique was applied, have simultaneously measured the emissions of all denitrification gases from soils (De Wever et al. 2002; Weier et al. 1993; Scholefield et al. 1997b), while others usually exclude NO, although this gas constitutes an obligatory intermediate product in the reaction sequence (Conrad 1996; Firestone and Davidson 1989; Russow et al. 2009). In this study, Michaelis-Menten kinetics fitted the dependence of the maximum N_2 or N_2O emissions from soil denitrification on initial NO_3^- levels very well (Fig. 2a–b), but the maximum NO emissions showed an inconsistent pattern (Fig. 2c). The maximum NO emissions was stimulated by the initial NO_3^- concentration at low levels (10–50 mg N kg^{-1} ds) but inhibited under conditions with higher initial NO_3^- levels (>50 mg N kg^{-1} ds) (Fig. 2c). Similar results

were reported by Ludwig et al. (2001), who found that the rate of NO emission not only depends on the overall denitrification rate but is also strongly affected by parameters that influence the proportion of NO relative to the terminal products of denitrification, i.e., N_2O and N_2. However, such a relationship between the peak NO emission and soil NO_3^- concentration could not be demonstrated by Shannon et al. (2011), who worked with *Pseudomonas mandelii* inoculated into anoxic soil. These authors did not observe any significant change in NO reductase activity during incubations with different starting concentrations of soil NO_3^- (>10 mg N kg^{-1} ds). The further study is still needed to explain the reason for the inhibitory effect of high initial NO_3^- concentration on NO emission from denitrification.

The maximum N_2 emissions observed in our treatments varied from 600 to 2300 μg N h^{-1} kg^{-1} ds. In comparison, previous studies using the gas-flow-soil-core technique for denitrification measurements have reported much higher values in some cases but lower values in others. For instance, Swerts et al. (1996a) observed 3.6-fold higher maximum N_2 emissions, i.e., 200 versus 55 mg N d^{-1} kg^{-1} ds (2300 μg N h^{-1} kg^{-1} ds) in our study, from a clay silt loam for the initial NO_3^- level of 100 mg N kg^{-1} ds. The higher maximum N_2 emissions were most likely due to the higher initial carbon substrate concentration (950 versus 300 mg C kg^{-1} ds in our study). Cárdenas et al. (2003) demonstrated maximum N_2 emissions that were one magnitude lower (2.6 versus 31 kg N ha^{-1} d^{-1} in our study) from a grassland soil with an initial NO_3^- content of 50 kg N ha^{-1} and DOC content of 360 kg C ha^{-1}, which were comparable to the nitrogen and carbon substrate concentrations in our 50 N treatment. The different magnitudes in peak N_2 emissions might have been caused by differences in the soil properties such as soil pH (5.7 versus 8.5 in our study).

Effect of initial NO_3^- concentrations on cumulative emissions of denitrification gases

In this study, the cumulative N_2 emissions showed a linear and positive dependence upon the initial NO_3^- concentrations (Fig. 2d). Scholefield et al. (1997b) have also observed a similar linear relationship for NO_3^- applications lower than 150 kg N ha^{-1} (approximately 150 mg N kg^{-1} ds). When the NO_3^- addition rate was further increased to 200 kg N ha^{-1} (approximately 200 mg N kg^{-1} ds), however, Scholefield and his colleagues observed a reduced N_2 production, which was inconsistent with our results (Fig. 2d).

Scholefield et al. (1997b) reported that the linear increase in N_2O emissions from denitrification correlated with the NO_3^- application rates of 0–200 kg N ha^{-1} (approximately 0–200 mg N kg^{-1} ds). We, however, observed an exponential increase between cumulative N_2O emission and initial NO_3^- concentration (Fig. 2e). The increase in N_2O emissions at very high NO_3^- concentration was likely caused by two mechanisms. One of these mechanisms was high NO_3^- inhibition of N_2O reductase (Blackmer and Bremner 1978). This mechanism would lead to a reduction in N_2 emissions, as reported in the literature (e.g., Ruser et al. 2006; Scholefield et al. 1997b), or a slowed-down increase in N_2 emissions. The latter effect could be seen in our study; at the highest NO_3^- application rate the N_2 emissions were only slightly higher compared to the 180 N treatment (Fig. 2d). The other mechanism was carbon substrate limitation of denitrification. In general, when the supply of the organic carbon substrate, as a reductant, prevails over the availability of oxidants, such as nitrate, complete denitrification occurs, and N_2 is the main product. Otherwise, carbon substrate limitation occurs, resulting in incomplete denitrification, with N_2O being generated as the main product (e.g., Hutchinson and Davidson 1993; Senbayram et al. 2011; Zumft 1997). This mechanism would easily explain the observed patterns of nitrogenous gas production at high soil NO_3^- concentrations, i.e., following the increase of incubation temperature to 25°C, a small N_2O peak followed by a high N_2 peak was observed. The N_2 peak was again followed by a second much higher N_2O peak before finally the N_2 production became the dominating end product of denitrification (Fig. 1f–g). With regard to the carbon substrate supply, this result can be interpreted as follows: at the beginning, the carbon substrate was not limiting, but the diffusion of NO_3^- to the sites of active denitrification started to become limiting, resulting in a peak of N_2 emissions. The second peak of N_2O emissions can therefore be interpreted as resulting from the carbon substrate becoming limited, which resulted in incomplete denitrification, since NO_3^- supply by diffusion was not limiting. Finally, the NO_3^- diminished, and at the reduced rates of denitrification, the carbon substrate supply was not limiting anymore. Consequently, N_2 was

the main end product of denitrification and not N_2O (Fig. 1f–g). However the above mentioned two mechanisms remain speculative since due to methodological restrictions we did not measure NO_3^- and DOC concentrations or enzyme activities during the incubation.

Molar ratios among denitrification gases and CO_2

We did not observed significant relationships between the molar ratios (1.7–5.3) of N_2:N_2O emissions from denitrification with initial soil NO_3^- concentrations. This finding differs from the results of Scholefield et al. (1997b), who observed an exponential decrease ($p<0.05$) in N_2:N_2O molar ratios (from 3.0 to 0.5, adapted from data in the literature) with increases in soil NO_3^- concentrations (from 25 to 200 kg N ha^{-1}, corresponding to approximately 25 to 200 mg N kg^{-1} ds). The ratios of N_2:N_2O for periodic emissions associated with denitrification were also measured in other previous studies that reported values of 0.2 to 2.8 for grassland soils (Cárdenas et al. 2003; Swerts et al. 1996a) and 1.7 to 3.5 for soils of cultivated lands (Schlesinger 2009; Senbayram et al. 2011). In comparison with the N_2:N_2O ratios associated with periodic emissions, the range of these ratios related to the instantaneous emissions were much wider. For instance, the N_2:N_2O ratios during the late anaerobic incubation period in our experiment were usually greater than 100, and ratios of 5–200 have also been reported for arable soils in Uzbekistan (Scheer et al. 2009) and forest soils in southern Germany (Dannenmann et al. 2008). These examples indicate that N_2:N_2O ratios can vary significantly with soil nitrate and carbon substrate availability, redox potential, soil properties, and denitrifier activity (e.g., Ciarlo et al. 2008; Weier et al. 1993; Philippot et al. 2011; Morley and Baggs 2010).

The mean NO:N_2O molar ratios of the cumulative emissions during anaerobic incubation conditions at 25°C were in the range of 1.0–1.5 for all of the treatments, excluding 180 N and 250 N, which showed lower ratios of <0.5. This shows that NO is a significant by-product of denitrification. Similar results were reported by Anderson and Levine (1986), who observed a NO:N_2O ratio of 3.0 in pure culture of the denitrifier *Alcaligenes faecalis* in a liquid medium. When the authors pure-cultured the denitrifiers *Rhizobium japonicum* and *Pseudomonas fluorescens* in the same medium, they observed NO:N_2O molar ratios less than 1.0. Due to the fact that in our experiment, even under strict anaerobic conditions, NO:N_2O molar ratios greater than 1.0 were commonly observed under conditions with low to moderate initial NO_3^- concentrations; therefore, earlier statements by different authors using the NO:N_2O ratio as an indicator to judge whether nitrogen trace gas fluxes are nitrification (NO:N_2O ratio >1) or denitrification (NO:N_2O ratio <1) dominated must be reconsidered (Del Prado et al. 2006; Scheer et al. 2009).

Theoretically, the molar ratios of the CO_2–C emissions to NO–N, N_2O–N or N_2–N during denitrification are 0.75 (NO), 1.00 (N_2O) or 1.25 (N_2) (Swerts et al. 1996a). The ratios we observed in the 80 N to 250 N treatments (0.9–1.1) are in good agreement with these theoretical values, whereas higher ratios (>1.9) were detected in other treatments with lower initial NO_3^- concentration. Swerts et al. (1996a) and De Wever et al. (2002) also observed comparably high ratios in anaerobic soil incubation. The higher ratios were likely caused by CO_2 production in anaerobic process other than denitrification, such as fermentation or microbial iron or sulfate reduction (Swerts et al. 1996a; Achtnich et al. 1995; Yao et al. 1999). The fact that those other microbial processes were significantly contributing to the CO_2 production can also be observed from the second peak of CO_2 emission in the 10 N treatment, since during this period the nitrogenous gas emissions were already close to the detection limit.

Nitrogen and carbon balances

The changes in soil NO_3^- pools between the beginning and end of the incubation for all treatments employed in this study were largely explained by the emissions of nitrogenous gases, with recovery rates ($N–RR_n$: 84 % on average) falling in the range of those reported in other studies, which varied from 42–57 % (Scholefield et al. 1997b) to 91–117 % (Swerts et al. 1996a). The emissions of nitrogenous gases explained the changes in the total soil nitrogen pool (i.e., the sum of NO_3^-, NH_4^+ and SMBN) slightly better ($p<0.01$), with an average recovery rate ($N–RR_f$) of 88 % being observed. In view of the uncertainties involved in measurements of soil NO_3^-, NH_4^+, SMBN and nitrogenous gas emissions from denitrification, these results were encouraging. The soil NH_4^+ contents significantly increased for all NO_3^- treatments. Increases in soil NH_4^+ concentrations during anaerobic soil incubation have also been reported by Scholefield et al. (1997b) and Meijide et al. (2010). This

indicates that under strict anaerobic conditions dissimilatory nitrate reduction to ammonia (DNRA) (Rütting et al. 2011) or anaerobic mineralization of organic matter (Bridgham et al. 1998) can play a significant role. For the former process N_2O was also produced (Rütting et al. 2011). Some studies have reported that N_2O production from DNRA accounted for 1 % (Cole 1988) to 5–10 % (Smith and Zimmerman 1981) of the NO_3^- amount, while the product NH_4^+ accounted typically for more than 90 % (Bleakley and Tiedje 1982). However, we are not able to finally judge if DNRA is indeed an important process for nitrogenous gas formation unless ^{15}N isotopes are used. This topic deserves further investigation.

The measured CO_2 emissions only explained 35–77 % of the losses of soil carbon pool (i.e., DOC plus SMBC). The carbon pool losses that were not from CO_2 emissions were attributed to three possible reasons. The first reason was likely fermentation-induced losses. This process could have occurred simultaneously or sequentially with denitrification under anaerobic conditions, competing for DOC and producing volatile fatty acids (Swerts et al. 1996a,b) that were not detected in this study. The second cause was likely due to CO_2 dissociation in soil water under conditions with high pH (Ingwersen et al. 2008). The final reason was likely underestimation due to no employing any correction for SMBC using an extraction coefficient (Joergensen 1996).

Conclusions

The dynamics and magnitudes of nitrogen gas (N_2), nitrous oxide (N_2O), nitric oxide (NO) and carbon dioxide (CO_2) emissions from denitrification in a silt clay calcic cambisol during anaerobic incubation were directly measured, using the gas-flow-soil-core technique that has been proven to be a useful and reliable tool to investigate emissions of N_2 and the other gases. In the treatments with different initial nitrate (NO_3^-) concentrations, almost all NO_3^- were consumed during anaerobic incubation, with 80–88 % of the changes in the soil NO_3^- pools being recovered by measuring the emissions of nitrogenous gases. The increases in initial NO_3^- concentration significantly enhanced the denitrification potential and the emissions of N_2O and N_2 as products of the process. The individual products of N_2, N_2O and NO accounted for the denitrification potential with very narrow fraction ranges in spite of the much wider range of initial NO_3^- concentrations. This study strongly supported the hypothesis that denitrification is significant for N_2O and NO emissions from calcic cambisols under conditions of sufficient supplies of carbon and nitrogen substrates and anaerobiosis. Our study provides some directly measured parameters for model-simulating the denitrification process of the investigated soil. In addition, the product ratios of NO: N_2O in denitrification were more than 1.0 when the initial NO_3^- concentrations were at low to moderate levels and vice versa. However, further study is needed to test whether these results are specific to calcic cambisols. To better parameterize the denitrification process of a soil, dynamic monitoring of carbon and nitrogen substrates during incubation is also strongly demanded in further studies using the gas-flow-soil-core technique.

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