Regulation factors of denitrification and their influence on emissions of N_2O from pasture soil

Regulační faktory denitrifikace a jejich vliv na emise N_2O v pastevních půdách

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Annotation:
Regulating factors of denitrification and their effect on emissions of N_2O from pasture soils in a cattle overwintering area were investigated. The study was based on field experiments performed at three locations along the gradient of animal impact and on laboratory experiments focused on effect of nutrients addition on N_2O emissions.

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Author´s contribution

Petr Brůček was responsible for preparing of treatments, sampling and completion of data including preparation of the manuscripts of the papers listed above.

Co-author´s agreement

We hereby declare that Petr Brůček had a major contribution of first paper from the list of papers. His contribution to other papers listed, especially to the second and the last one, was also significant.

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Contents

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General introduction

Nitrous oxide (N_2O) belongs to important trace gases in the atmosphere. Similarly to other atmospheric gases, continual exchange of N_2O between atmosphere and soil takes place. Soil is both the source and the sink of N_2O and it is produced (and consumed) during biological processes of nitrogen (N) transformations. These processes include especially nitrification and denitrification. Intensive human activity that is responsible for changes in natural and anthropogenic ecosystems strongly affects also transformations of nutrients in soil. Besides other effects, it increased significantly emissions of N_2O and consequently atmospheric concentration of N_2O. Nitrous oxide is not toxic and it is relatively inert, but it plays important role in atmospheric chemistry participating in reactions which are responsible for destruction of atmospheric ozone. And finally it belongs to so called greenhouse gases, which cause global warming of the atmosphere. Although the relationship between concentration of N_2O in the atmosphere and global temperature is not simple, its existence is supported by data on concentration of N_2O in the air entrapped in Antarctic ice – samples from layers from warmer periods contained higher amount of N_2O in comparison with layers deposited in colder conditions (Smith 1997).

Production of N_2O is one of the processes of nitrogen losses from the soil and it is accompanied by other undesirable processes (nitrate leaching, production of NO) that also have serious environmental impacts (excessive input of N into natural ecosystems, high concentration of nitrate in water etc.). These negative effects make N_2O emissions an important process that has been intensively studied in recent decades. However, amount of N_2O emitted from the soil and factors affecting N_2O emissions are not yet fully clear.

Importance of pastures as a source of N_2O is supported by numerous publications (e.g. Christensen 1983; Jarvis, Pain 1997). High amount of available carbon, nitrogen and other nutrients from animal excrements support intensive microbial transformations of N including N_2O producing processes. Thus, pasture soils represent a suitable environment for study of factors affecting production of N_2O in and emissions from the soil. Improved knowledge of processes producing N_2O may contribute to better management of soils with respect to environmental impacts.

Processes producing N_2O in soil

Nitrous oxide is produced during redox transformations of N in soil. The main processes of its production are denitrification and nitrification (Skiba et al.

1992; Bremner 1997), assimilative and dissimilative reduction of nitrate (NO_3^-) to ammonium (NH_3) and biological fixation of atmospheric N_2.

Denitrification

Denitrification is process of NO_3^- reduction to N_2 through NO and N_2O as intermediate products. It may proceed as chemical reaction called chemodenitrification or biological process of anaerobic respiration that uses NO_3^- as electron acceptor instead of oxygen (O_2). Biological denitrification is usually mentioned to be main process of nitrate reduction while importance of chemodenitrification is low (Paul, Clark 1996; Killham 1994).

Biological denitrification needs specific conditions:
1) decreased concentration of O_2 - molecular O_2 is better electron acceptor than NO_3^- and its presence in soil inhibits denitrification; the highest production of N_2 and N_2O takes place when pores are filled with water, at low porosity or in conditions of O_2 exhaustion by intensive respiration,
2) presence of sufficient amount of NO_3^- - it is produced through mineralization and nitrification of organic N, in agricultural soils it is often added in the form of mineral or organic fertilizers,
3) source of available C – for example in the form of dead plant tissues, dead microbial biomass, organic fertilizers.

Denitrification is natural process that returns N, previously taken from the atmosphere as N_2 during biological fixation, back to the atmosphere. Final product of the process is N_2, but reduction is often imperfect and significant amount of N_2O is released. Total amount and ratio of these gaseous products are variable and it is regulated by many factors (Paul, Clark 1996).

Denitrification is the sequence of enzymatic reactions converting oxidized nitrogen species to most reduced one:

$$\mathbf{NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2}$$

Individual steps of denitrification are regulated by environmental conditions. Production of N_2O is limited by the rate of the last reaction – reduction of N_2O to N_2. When reduction of NO_3^- and NO_2^- proceeds in higher rate than reduction of N_2O, accumulation of N_2O takes place. The rate of reduction of N_2O to N_2 is affected especially by content of available C, ratio C : NO_3^- and concentration of O_2 in soil air (Coyne 1999). At lower ratio C : NO_3^-, reducing agent becomes limiting and sequence of reactions ends by release of N_2O. In contrast, higher ratio enables perfect reduction to N_2 (in certain conditions, soil can consume and reduce atmospheric N_2O) (Mosier, Schimel 1991; Simarmata et al. 1993). Majority of typical bacterial denitrifiers has ability of aerobic respiration (O_2 is better oxidant than NO_3^-); therefore the increasing content of O_2 in soil decreases ratio N_2:N_2O and inhibits denitrification.

Generally, denitrifying enzymes are activated in the following order: NO_3^- - reductase, NO_2^- - reductase, N_2O – reductase, and inhibited in the reverse order. It means that resulting proportion of N_2O is affected by ratio and also duration of suggested regulating factors (Dendooven et al. 1997; Priemé and Christensen 1991).

Nitrification

Nitrification is another important source of N_2O in soil. Nitrification is microbial oxidation of NH_4^+ to NO_3^- by O_2. It proceeds in two steps:
- 1st phase (nitritation) - NH_4^+ is oxidized to NO_2^-
- 2nd phase (nitratation) - NO_2^- is further oxidized to NO_3^-

During both steps, N_2O may be released as by-product. Significance of nitrification as N_2O producing process was documented for example in experiments where soil fertilized by urea or $(NH_4)_2SO_4$ produced higher amount of N_2O than soil fertilized by NO_3^- (Bremner 1997).

There are several possibilities, how nitrification may contribute to N_2O production:
- N_2O is produced during oxidation of NH_4^+ to NO_3^-
- nitrification produces NO_3^- that is further denitrified
- the 2nd phase of nitrification (nitratation) is more sensitive to environmental conditions than the 1st one (nitritation). In stressful conditions nitratation may be stopped and NO_2^- accumulates that is reduced to N_2O. It may be the case in extreme environment, for example in urine patches in pastures where toxic effect of high concentration of NH_4^+ on nitrifiers takes place (Oenema et al. 1997),
- denitrifying nitrification - NO_2^- is utilized for oxidation of NH_4^+ (Nielsen et al. 1996).

Factors affecting production of N_2O

Content and form of N in soil

Most of N_2O in soil is produced from NO_3^- and NO_2^- during denitrification or from NH_4^+ during nitrification. In soils with intensive N transformations, most of NH_4^+ released through mineralization is oxidized to NO_3^-. Ammonium N only accumulates at low temperatures (Killham 1994) and during dry periods when activity of nitrifiers is limited (Paul, Clark 1996). Content of NO_2^- in soil is usually low and it accumulates only in extreme conditions. Soils where NH_4^+ form of N prevails have usually lower rates of N transformations and lower emissions of N oxides (Davidson, Verchot 2000).

Effect of NO_3^- on the rate of denitrification is not simple. At relatively low content of NO_3^-, (for example Webster and Goulding (1989) report 2 - 5 mg N kg^{-1}) denitrification runs as zero order reaction, the rate is independent on NO_3^- concentration and it is limited by availability of C (Ottow et al. 1985). This finding is supported by results of field experiments where intensive denitrification was determined even at low level of soil NO_3^- (Teepe et al. 2000).

In contrast, relationship between NO_3^- content and production of N_2O and increased emissions of N_2O following input of fertilizer was often reported (Jarvis, Pain 1997; Christensen 1983).

Content of available C and its sources in pasture soils

Production of N_2O by respiration denitrification needs sufficient amount of organic C. Input of N in organic form usually stimulates emissions in higher rate than addition of comparable amount of N in the form of mineral fertilizer (Christensen 1983).

Carbon is not only donor of electrons during reduction of NO_3^-; it also enables growth of microbial biomass that performs denitrification. In addition, high consumption of O_2 and production of CO_2 during periods of intensive microbial activity creates anaerobic conditions necessary for denitrification (Parkin 1987). On the other hand, increased growth of biomass also consumes mineral nitrogen.

The source of C in pasture soil may be plant residues, root exudates, organic fertilizers, excrements of animals and also dead microbial biomass. Important factor that affects availability of C in soil for microorganisms is not only quantity, but also quality of substrate, especially ratio C:N and solubility.

In different periods of the year, different sources of C in pasture ecosystems are available. In spring during periods of freezing and thawing C is released from dead microbial biomass. Therefore early spring is usually considered as typical period of increased N_2O fluxes. During vegetation, C from root exudates is utilized (Killham 1994). At the end of vegetation plant residues become the source of C that may be responsible for profound and long-term increase of N_2O emissions (Beck, Christensen 1987).

Soil water content

Very important regulation factor of denitrification is soil moisture. It affects production and emissions of N_2O through several ways. Soil water regulates soil aeration status, availability of carbon and nutrients and release of N_2O from soil. It is often expressed as water filled pore space (WFPS). It means the volume of soil pores actually filled with water. When WFPS increases, volume of air in soil decreases. At WFPS higher than 60% anaerobiosis can take place and microbes start to use other electron acceptors than O_2. This situation provides suitable conditions for denitrification.

Water is also transporting medium for NO_3^- in soil. Up to some limit, it increases availability of NO_3^- for soil microorganisms. When water content is too high, for example after heavy rain, NO_3^- may be leached from the soil in higher rate than it is denitrified (Jarvis, Pain 1997). N_2O is also produced by nitrification that is limited by O_2 at high WFPS and proportion of N_2O from nitrification decrease at high water content. On the other hand, when content of water is too low, diffusion of substrates to microbial cells is limited and it decreases also N_2O emissions (Davidson 1993).

N_2O in soil may be dissolved in soil water. It causes its storage in soil or transport through the soil (Heincke, Kaupenjohann 1999). It can be leached from the soil that causes biases in estimates of N_2O production (Rice, Rogers 1993). When N_2O is stored in soil for sufficient time, it may be reduced to N_2 (Robertson 1993; Heincke, Kaupenjohann 1999).

Not only actual water content, but also its short term changes are important driving factor for N_2O emissions. Precipitation or irrigation often stimulates denitrification and rate of N_2O production exceeds the rate of N_2O reduction to N_2. When rapid drying takes place, denitrification stops and unreduced N_2O is released. On the other hand, the main product of denitrification may become N_2 when soil is water saturated (Priemé, Christensen 1991).

Soil aeration status and O_2 content

Nitrous oxide is produced by nitrification and denitrification. These processes have contrasting demands for aeration. Thus, the effect of aeration on emissions may be unpredictable. Production of N_2O by nitrification usually increases with increasing content of O_2 in soil. In contrast, production of N_2O by denitrification increases when diffusion of O_2 slows down. However, diffusion of produced N_2O also decreases at the same time, microbial demands for electron acceptors increases and N_2O reduction to N_2 is stimulated (Priemé, Christensen 1991). Content of molecular O_2 in soil air is important regulation factor of denitrification and on set-up of final products from denitification. Presence of O_2 prevent synthesis of denitrification enzymes. The most sensitive enzyme is enzyme responsible for alteration of N_2O to N_2. If there are higher concentrations of O_2, $N_2O:N_2$ ratio is raised while the rate of denitrification declines.

Rate of N_2O emissions is strongly regulated by the rate of gas exchange between soil and atmosphere. In soil, gases move especially by diffusion along the gradient of concentrations (Hillel 1998). Gases produced in soil, for example CO_2, N_2O or CH_4 diffuse into the atmosphere, where their concentration is lower. O_2 moves in opposite direction from the atmosphere to soil. Gas exchange in soil is often impaired and soil products accumulate while O_2 content is limited.

Important factor of aeration in pasture soils is compaction of soil by animal movement and trampling. Compaction decreases volume of pores which helps to create anaerobic conditions suitable for denitrification (Oenema et al. 1997).

Aims and research questions

General hypothesis: Emissions of N_2O at overwintering area are supported by large inputs of nutrients from cattle excrements. Cattle impact not only stimulates actual emissions, but it has also long-term effect on potential production of N_2O. Besides total amount, proportions of nutrients regulate production (and consumption) of N_2O. Reduction of N_2O to N_2 is higher in soil with higher impact of cattle. Actual rates of emissions are affected by environmental conditions.

We hypothesized that emissions are proportional to inputs of N. Increasing nutrient input along the gradient of cattle impact increases not only actual production of N_2O following nutrient input, but it also increases potential production of N_2O. Amount and ratio of C and NO_3^- amendments control both total amount and ratio of the two denitrification products, N_2O and N_2. Reduction of N_2O to N_2 is stronger in soil at the location most impacted by cattle and it decreases emissions of N_2O. Emissions are strongly controlled by temperature and physical properties of soil.

An overwintering area was selected at Borová Farm near Český Krumlov in Southern Bohemia (latitude 48°52´ N, longitude 14°13´ E), about 170 km South from Prague. The area was approximately 4 ha large, and it had been used for overwintering of about 90 cows since 1995. The animals were present on the site usually from November to May. Soon after the arrival of animals there was a visible gradient of animal impact from the most impacted areas near the animal house through much less impacted areas in the middle to almost unaffected areas at the opposite side of the overwintering area, where cattle traffic was minimal; these differences were most pronounced by the end of the winter season. Along this gradient three locations were identified, differing in the presumed rate of animal impact. These included a severely impacted location (S), with totally destroyed plant cover and surface soil, a location with moderate impact (M), where effects of trampling and disturbance of the vegetation were still visible, and a control location (C), with slight or no impact as judged from soil and vegetation.

Field and laboratory experiments were performed to determine potential production of N_2O by denitrification. These experiments were completed with laboratory determination of potential denitrification at various amounts and ratio of added C and N (Paper I). Effect of increasing input of N into soil was investigated in experiments with artificial application of different amounts of nutrients (Paper II). Ratio of denitrification products N_2 and N_2O at three locations along the gradient was determined in experiment using ^{15}N (Paper III). The aim of experiments was to determine relationship between inputs of nitrogen and emissions of N_2O. Short-term variation of N_2O emissions was investigated in several field measurements with the aim to find whether daily changes of temperature affect N_2O emissions (Paper IV). Two year measurements of emissions and related parameters were performed on studied area aiming to estimate annual emissions of N_2O at three differently impacted soils along the gradient (Paper V). Soil properties at locations of overwintering area differently impacted by cattle were investigated. At the same time, emissions of N_2O and CO_2 were measured with the aim to relate soil parameters to the rate of emissions (Paper VI).

Results

Paper I presents results of laboratory determination of potential denitrification using various amounts and ratios of C and NO_3^-. The relationships between the level of long-term animal impact and potential production of N_2O from soil by denitrification were investigated in field and laboratory experiments. Field measurements indicated that the production of N_2O after glucose and nitrate amendments was greater in severely and moderately impacted locations than in an unimpacted location, while differences between the severely and moderately impacted locations were not significant. In laboratory experiments, the potential production of N_2O (measured as anaerobic production of N_2O after addition of glucose and nitrate) was highest in the moderately impacted soil. Surprisingly, potential N_2O production was lower in the most impacted than in the moderately impacted soil, and the net N_2O production in the highly impacted soil was further decreased by a significant reduction of N_2O to N_2. The expected stimulating effect of an increasing ratio of glucose C : nitrate N on the reduction of N_2O to N_2 during denitrification was not confirmed. The results show that cattle increase the denitrification potential of the soil but suggest that the denitrification potential does not increase indefinitely with increasing cattle impact.

Paper II is targeted on field measurement of N_2O emissions affter application of high batch of C (glucose) and N (nitrate). The aims of this study were to: 1) experimentally quantify the relationship between mineral N input and N_2O emissions from denitrification; 2) describe the time course of N_2O fluxes resulting in N inputs; and 3) find whether there exists an upper limit of the amount of nitrogen escaping the soil in the form of N_2O. The study site was a grassland used as a cattle overwintering area. It was amended with KNO_3 and glucose corresponding to 10 - 1 500 kg N and C ha^{-1}, covering the range of nutrient inputs occurring in real field conditions. Using manual permanent chambers, N_2O fluxes from the soil were monitored for several days after the amendments. Peak N_2O emissions were up to 94 mg N_2O-N m^{-2} h^{-1} 5-8 hours after amendment. No upper limit of N_2O emissions was detected as the emissions were directly related to the dose of nutrients in the whole range of amendments used, but the fluxes reflected the soil and environmental conditions, too. Thus, in 3 different experiments performed during the season, total cumulative losses of N_2O-N ranged from 0.2 to 5.6% of the applied 500 kg NO_3^--N ha^{-1}. Splitting of high nutrient doses lowered the rate of N_2O fluxes following the first amendment, but the effect of splitting on the total amount of N_2O-N released from the soil was insignificant, as the initial lower values of emissions in the split variants were compensated for by a longer duration of gas fluxes. The results suggest that the cattle impacted soil has the potential to metabolize large inputs of mineral nitrogen over short periods (~days). Also, the emission factors for NO_3^--N did not exceed values reported in literature.

Paper III describes results of field determination of emissions of N_2 and N_2O in situ in three different sites along the gradient of animal impact. The ^{15}N gas-flux method was used to measure the emissions of N_2O and N_2 at three sites along a gradient of animal impact. Over the experimental period (72 h), the loss of NO_3^--N as N gases was 60, 12 and 3%, and the mole fraction of N_2O was 0.04, 0.15 and 0.75 for the severe, moderate and control treatments, respectively. We hypothesize that soil pH which is enhanced in impacted sites (from 5.7 up to 7.8) may control mole fraction of N_2O in such a way that under alkaline conditions most of the nitrogen escapes as N_2.

Paper IV is targeted on field measurement of spatial and temporal variability of N_2O emissions. Production of both N_2O and CO_2 changed quickly over a relatively short time, but the general course of fluxes of the two gases was different. CO_2 emissions were basically controlled by temperature and most chambers showed the same trend of flux development. In contrast, emissions of N_2O were not only extremely variable, but each chamber had its own time course of emissions; therefore the relationship between N_2O fluxes and temperature was far more complicated. According to our results, we strongly recommend detailed investigations including frequent emission measurements in periods of high gas fluxes as the way of more precise estimations of gas emissions over longer periods.

Paper V contains results of field measurements of N_2O emissions. Emissions of N_2O and CO_2 from a cattle overwintering area were measured during two years. The measurements were performed at three sampling locations along a gradient of animal impact (severe, moderate, slight) to test the hypothesis that emissions of CO_2 and N_2O are positively related to the degree of impact. In addition to CO_2 and N_2O fluxes, soil mineral nitrogen (NH_4^+ and NO_3^-), pH and temperature were determined to assess possible regulations of gas fluxes. Deposition of animal excreta resulted in a significant accumulation of nitrogen in the soil during winter. Most of the N_2O was emitted during a few short periods in spring and/or in late autumn. Large N_2O fluxes were associated with recent rainfall on some sampling dates. During winter and spring, presumably in periods of increasing temperatures, intensive soil nitrogen transformations took place, followed by peaks of N_2O emissions. Maximum N_2O fluxes of up to 2.5 mg $N\text{-}N_2O\ m^{-2}\ h^{-1}$ were usually recorded at the most impacted location near the animal house, where also the highest concentrations of mineral nitrogen occurred. However, the effect of animal impact was not simple and on some occasions N_2O emissions were higher at the moderately impacted location. The emissions of CO_2 showed a completely different pattern than those of N_2O, being correlated with soil temperature; the highest emissions thus occurred in June-July, while very low fluxes were found in winter. Emission values ranged from about zero to 700 mg $C\text{-}CO_2\ m^{-2}\ h^{-1}$. Further, the effect of animal impact on CO_2 emissions was opposite to that on N_2O fluxes, as the highest CO_2 fluxes were mostly recorded at the least impacted location, where likely respiration of plants increased overall CO_2 production. The potential for N_2O reduction to N_2 was determined in severely and moderately impacted soil in early autumn using acetylene inhibition; N_2 was generally the main nitrogen gas emitted. To test the relationship between rainfall and N_2O emissions, experimental wetting of dry soil was performed which caused a sharp, but short-lived increase of N_2O emissions.

Paper VI contains results describing transect with different cattle impact at overwintering area. Fluxes of N_2O and CO_2 were measured and physical and chemical parameters of pasture soil at the area were determined. Excretal returns and physical disturbance due to treading can greatly influence nitrogen flows in grazed pastures. Dung and urine depositions stimulate microbial transformations, while soil compaction and poaching change the physical environment in which these transformations take place. In this study, a cattle overwintering area in the Southwest Czech Republic was characterized with respect to bulk density, porosity, water-filled pore space (WFPS), organic C, total N, pH, microbial biomass C and denitrifying enzyme activity (DEA). Carbon dioxide and nitrous oxide (N_2O) emissions were measured on four different dates between October 2001 and May 2002. Sampling took place along a transect away from an open barn with access to feed. Soil chemical and biological properties showed that deposition of excreta declined with distance from the barn. In contrast, N_2O emissions were highest at intermediate positions along the transect. At the section with the greatest animal impact, the ratio of N_2 versus N_2O produced was five-fold higher, and the soil pH was 2 units higher, compared to the section with the least animal impact, which indicated that soil conditions favoured production of N_2 rather than N_2O in the area where excretal returns and treading was intense. A multiple linear regression was conducted using data from the last sampling. There were significant effects of WFPS and pH on log-transformed N_2O emissions, while effects of NH_4^+ and NO_3^-, and interactions between NH_4^+ and, respectively, WFPS and pH were nearly significant. The observations indicate that, whereas pasture management to achieve a better distribution of animal impact may improve N retention in the soil, it is not clear whether this will reduce N_2O emissions.

General discussion and conclusions

The main regulating factor of N_2O emissions is the rate of nutrients input. It was confirmed during two years of regular measurement of emissions. Accumulation of carbon and nitrogen during winter creates favourable conditions for high production of N_2O and annual production of N_2O was higher in soil with higher annual input of N (Paper V). Moreover, emissions of N_2O in cattle-impacted soil may be further stimulated by addition of available C and nitrate N and soil at the cattle overwintering area is able to produce large quantities of N_2O during short-term bursts of activity following inputs of nutrients (Papers I, II, III and IV). Emissions of N_2O measured during these bursts are proportional to the amount of added mineral nitrogen. Increasing doses of nutrients increased cumulative values of emissions proportionally to the level of fertilization without any limit in a broad range of nitrate-nitrogen inputs (0 – 1 500 kg N ha^{-1}) (Paper II). The experimentally estimated losses of nitrogen in the form of N_2O were in the range of 0.2 – 5.6 % of applied nitrogen, which is in the range of previously reported values (Velthof et al. 1997; Fowler et al. 1997; Lampe et al. 2006; McTagart et al. 1997).

Long-term history of cattle impact increases not only actual rates of N_2O production, but also the potential production of N_2O from the pasture soil (Paper I). Denitrification potential, however, does not increase indefinitely with increasing cattle impact despite higher nutrient inputs and accelerated microbial activities. Besides higher rate of denitrification, increasing cattle impact stimulates reduction of N_2O to N_2. It was recently confirmed in experiment by Chroňáková et al. (2009) who observed increased activity of denitrifying enzymes along the gradient of cattle impact with N_2 as prevailing terminal product in soil with the highest effect of cattle. Similar results were also observed in field experiment with addition of $^{15}NO_3^-$ (Paper III). In contrast, different picture showed laboratory measurement of potential denitrification. The highest potential production of N_2O was determined in soil with moderate cattle impact. Reduction of N_2O to N_2 only partly explains why N_2O emissions were not greater in the severely than in the moderately impacted soil because total denitrification potential was lower in the severely impacted than in the moderately impacted soil. At the highest level of cattle impact, other N consuming processes (assimilatory or dissimilatory reduction of N rather than denitrification) or the accumulation of deleterious intermediate products after amendments probably reduced the potential production of N_2O.

The amount of N_2O released from the soil is also strongly affected by soil and environmental conditions, which are likely to include soil temperature and moisture and porosity (Papers IV, V and VI). In field measurements targeted on the short-term effects of temperature on emissions of N_2O and CO_2, CO_2 was produced continually in the soil and its emission was often correlated with temperature. On the other hand, the pattern of N_2O flux was much less predictable than that of CO_2 (Paper IV). It is contrary to laboratory measurements of potential denitrification where production of CO_2 and N_2O were correlated (Paper I). In addition, close correlation between CO_2 and N_2O emissions was found in field experiment with measurement of emissions from soil amended with high input of C and NO_3^-. This discrepancy suggests that in field conditions, N_2O is produced in limited space and time. At the overwintering area, inputs of nutrients take place during long period from November to May and each microsite producing N_2O receives input of nutrients in different time. Production of N_2O is characterized by sharp, short-term peaks of fluxes at each microsite and in result, emissions are heterogeneous in space and time (Paper IV). Obviously, the estimates of gas production and cumulative fluxes in general and those of N_2O in particular must be based on very detailed knowledge of diurnal variations in fluxes in the given period. Our results also suggest detail investigation of emissions during period of increased fluxes as possible way of better estimation of annual fluxes of N_2O.
Estimation of relationships between physical soil parameters other than temperature and N_2O emissions do not enable clear conclusions (Papers V and VI). The effect of rainfall and soil moisture is very complex and difficult to assess. In our conditions, the highest flux of N_2O emissions was observed shortly after rainfall. However, high fluxes were also determined in periods of dry early spring (Paper V).

Results of simultaneous measurements of N_2O emissions with determination of porosity suggest effect of physical conditions regulating aeration status. However, a hypothesis that lower porosity of soil with higher cattle impact increases emissions of N_2O was not supported by experimental data (Paper VI).

This is in agreement with controversial results reported in other studies. Decreased porosity or increased water-filled pore space not only stimulate denitrification, but they also accelerate reduction of N_2O to N_2 (Priemé, Christensen 1991). Results of diurnal measurements suggest that emissions may change rapidly and it may also rapidly change their relationship with other environmental factors. With available methods, these changes are hard to follow. In addition, effect of cattle on soil physical properties is not simple and it depends on interaction with other environmental factors. Cattle trampling not only leads to compaction, but it also creates patches of loosened soil. Soil surface is also very rough and heterogeneous that makes determination of physical parameters difficult. Despite that, the data presented in Papers I - VI improve the knowledge on N-transformations in pasture soils as well as on regulatory role of some soil and environmental factors on rates on N_2O formation in, and emission from, the soil.

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I

Long-term animal impact modifies potential production of N_2O from pasture soil

Brůček, P., Šimek, M., Hynšt, J., 2009. Long-term animal impact modifies potential production of N_2O from pasture soil. Biology and Fertility of Soils 46, 27-36.

Podíl na publikaci 60%

Abstract

At cattle overwintering areas, inputs of nutrients in animal excrements create conditions favourable for intensive microbial activity in soil. During nitrogen transformations, significant amounts of N_2O are released, which makes overwintering areas important sources of N_2O emission. In previous studies, however, increasing intensity of long-term cattle impact did not always increase emissions of N_2O from the soil: in some cases, N_2O emissions from the soil were lower at the most impacted area than at the moderately impacted one. Thus, the relationships between the level of long-term animal impact and potential production of N_2O from soil by denitrification were investigated in field and laboratory experiments. Field measurements indicated that the production of N_2O after glucose and nitrate amendments was greater in severely and moderately impacted locations than in an unimpacted location, while differences between the severely and moderately impacted locations were not significant. In laboratory experiments, the potential production of N_2O (measured as anaerobic production of N_2O after addition of glucose and nitrate) was highest in the moderately impacted soil. Surprisingly, potential N_2O production was lower in the most impacted than in the moderately impacted soil, and the net N_2O production in the highly impacted soil was further decreased by a significant reduction of N_2O to N_2. The expected stimulating effect of an increasing ratio of glucose C : nitrate N on the reduction of N_2O to N_2 during denitrification was not confirmed. The results show that cattle increase the denitrification potential of the soil but suggest that the denitrification potential does not increase indefinitely with increasing cattle impact.

Abstrakt

V půdě zimoviště skotu vytvářejí vysoké vstupy živin příznivé podmínky pro intenzivní mikrobní aktivitu. Během mikrobních transformací vzniká značné množství N_2O a proto je zimoviště významným zdrojem emisí N_2O. Nicméně v předchozích měřeních bylo zjištěno, že rostoucí vliv skotu nemusí být vždy spojen s nárůstem emisí N_2O a v několika případech byly emise z půdy nejvíce zatížené skotem nižší než emise z půdy se střední zátěží. Proto byla závislost mezi dlouhodobým vlivem skotu a potenciální produkcí N_2O denitrifikací sledována v polních a laboratorních pokusech. Z polních měření vyplývá, že produkce N_2O po přídavku glukózy a nitrátu byla větší v půdě středně a silně zatížené než v půdě neovlivněné, ale rozdíly mezi silně a středně zatíženou půdou byly neprůkazné. V laboratorních pokusech bylo zjištěno, že potenciální produkce N_2O (měřena jako anaerobní produkce N_2O po přídavku glukózy a nitrátu) byla nejvyšší v půdě středně zatížené. Potenciální produkce N_2O byla překvapivě nižší v nejvíce zatížené půdě než ve středně zatížené a čistá produkce N_2O v nejvíce ovlivněné půdě byla dále snižována redukcí N_2O na N_2. Předpokládaný stimulační efekt rostoucího poměru C : NO_3^- na redukci N_2O na N_2 během denitrifikace nebyl potvrzen. Z výsledků vyplývá, že rostoucí vliv skotu zvyšuje potenciální denitrifikaci v půdě, ale potenciální denitrifikace neroste donekonečna s rostoucím vlivem zvířat.

II

Nitrous oxide emissions from cattle-impacted pasture soil amended with nitrate and glucose

Hynšt, J., Šimek, M., Brůček, P., 2007. Nitrous oxide emissions from cattle-impacted pasture soil amended with nitrate and glucose. Biology and Fertility of Soils 43, 853-859.

Podíl na publikaci 35%

Abstract

There is little information concerning N_2O fluxes in the pasture soil, which has received large amounts of nutrients as urine and dung for several years. The aims of this study were to: 1) experimentally quantify the relationship between mineral N input and N_2O emissions from denitrification; 2) describe the time course of N_2O fluxes resulting in N inputs; and 3) find whether there exists an upper limit of the amount of nitrogen escaping the soil in the form of N_2O. The study site was a grassland used as a cattle overwintering area. It was amended with KNO_3 and glucose corresponding to 10 - 1 500 kg N and C ha^{-1}, covering the range of nutrient inputs occurring in real field conditions. Using manual permanent chambers, N_2O fluxes from the soil were monitored for several days after the amendments. Peak N_2O emissions were up to 94 mg N_2O-N m^{-2} h^{-1} 5-8 hours after amendment. No upper limit of N_2O emissions was detected as the emissions were directly related to the dose of nutrients in the whole range of amendments used, but the fluxes reflected the soil and environmental conditions, too. Thus, in 3 different experiments performed during the season, total cumulative losses of N_2O-N ranged from 0.2 to 5.6% of the applied 500 kg NO_3^--N ha^{-1}. Splitting of high nutrient doses lowered the rate of N_2O fluxes following the first amendment, but the effect of splitting on the total amount of N_2O-N released from the soil was insignificant, as the initial lower values of emissions in the split variants were compensated for by a longer duration of gas fluxes. The results suggest that the cattle impacted soil has the potential to metabolize large inputs of mineral nitrogen over short periods (~days). Also, the emission factors for NO_3^--N did not exceed values reported in literature.

Abstrakt

Existuje jenom málo poznatků o emisích N_2O z půd na pastvinách dlouhodobě ovlivněných velkými vstupy živin ve formě moči a exkrementů zvířat. Cílem

práce bylo (1) experimentálně zjistit závislost mezi vstupem dusíku a množstvím N_2O, které vzniká při denitrifikaci, (2) popsat časový vývoj emisí N_2O, které vznikají v důsledku vstupu živin a (3) najít, jestli existuje horní hranice emisí N_2O. Pokusy probíhaly na travním porostu využívaném jako zimoviště skotu. Do půdy byl přidán KNO_3 a glukóza v množství odpovídajícím 10 – 1500 kg N a C na hektar, což je možný rozsah vstupu živin v reálných podmínkách. Emise byly monitorovány s využitím přenosných komor v průběhu několika dní po přídavku. Nebyla zjištěna horní hranice emisí N_2O, emise byly úměrné množství aplikovaných živin v celém rozsahu dávek. Celkové ztráty N_2O-N byly v rozsahu 0,2 – 5,6% aplikované dávky dusíku. Dělení dávek živin snížilo emise N_2O po prvním přídavku, ale vliv dělení na celkové množství N_2O uvolněného z půdy bylo neprůkazné, protože nižší počáteční hodnoty byly kompenzovány delším trváním emisí. Z výsledků vyplývá, že půda ovlivněná působením zvířat má velkou schopnost transformovat velké množství minerálního dusíku v krátké době. Emisní faktory pro NO_3^--N nepřekročily hodnoty uváděné v literatuře.

III

Gaseous nitrogen losses from a grassland area used for overwintering cattle

Šimek, M., Stevens, R.J., Laughlin, R.J., Hynšt, J., Brůček, P., Čuhel, J., Pietola, L., 2006. Gaseous nitrogen losses from a grassland area used for overwintering cattle. In: Soliva, C. R., Takahashi, J., Kreuzer, M. (Eds.). Greenhouse gases and animal agriculture: an update. International Congress Series 1293, 343-346.

Podíl na publikaci 10%

Abstract

The rates of N_2O emission at overwintering area were mostly directly related to the intensity of animal impact. However, laboratory measurements had shown a much greater potential for N_2O production from soil in a severely impacted site than indicated by field measurements, possibly due to factors affecting the mole fraction of N_2O. The ^{15}N gas-flux method was used to measure the emissions of N_2O and N_2 at three sites along a gradient of animal impact. Over the experimental period (72 h), the loss of NO_3^--N as N gases was 60, 12 and 3%, and the mole fraction of N_2O was 0.04, 0.15 and 0.75 for the severe, moderate and control treatments, respectively. We hypothesize that soil pH which is enhanced in impacted sites (from 5.7 up to 7.8) may control mole fraction of N_2O in such a way that under alkaline conditions most of the nitrogen escapes as N_2.

Abstrakt

Bylo zjištěno, že trvalé travní porosty využívané k přezimování skotu jsou významným bodovým zdrojem N_2O díky utužení půdy a akumulaci exkrementů. Hodnoty emisí byly úměrné vlivu zvířat, ale výsledky laboratorních měření naznačují mnohem vyšší potenciální produkci N_2O z půdy nejvíce zatížené zvířaty než bylo zjištěno v polních měřeních. Možným vysvětlením je působení faktorů, které ovlivňují molární poměr N_2O. Emise N_2O a N_2 byly měřeny s využitím ^{15}N na třech stanovištích podél gradientu vlivu skotu. Během měření (72 hodin) dosahovaly ztráty NO_3^--N ve formě plynů 60, 12 a 3% a molární poměr N_2O 0,04, 0,15 a 0,75 na silně zatížené, středně zatížené a kontrolní ploše. Tento rozdíl mohl být způsoben zvýšenou hodnotou pH na stanovištích ovlivněných zvířaty. Zvýšená hodnota pH působí na molární poměr N_2O tak, že větší podíl dusíku uniká ve formě N_2.

IV

Diurnal fluxes of CO_2 and N_2O from cattle-impacted soil and implications for greenhouse gases emission estimates over longer periods

Šimek, M., Brůček, P., Hynšt, J. Diurnal fluxes of CO_2 and N_2O from cattle-impacted soil and implications for greenhouse gases emission estimates over longer periods. Submitted.

Podíl na publikaci 35%

Abstract

Short-term diurnal changes of emissions of CO_2 and N_2O were determined in cattle overwintering area during several specific periods of the year. Production of both N_2O and CO_2 changed quickly over a relatively short time, but the general course of fluxes of the two gases was different. CO_2 emissions were basically controlled by temperature and most chambers showed the same trend of flux development. In contrast, emissions of N_2O were not only extremely variable, but each chamber had its own time course of emissions; therefore the relationship between N_2O fluxes and temperature was far more complicated. According to our results, we strongly recommend detailed investigations including frequent emission measurements in periods of high gas fluxes as the way of more precise estimations of gas emissions over longer periods.

Abstrakt

Krátkodobé diurnální změny v emisích CO_2 a N_2O byly zjištěny na zimní pastvině pro skot během několika charakteristických období roku. Produkce N_2O i CO_2 se mění velmi rychle i během relativně krátké doby, ale i základní průběh emisí těchto dvou plynů byl rozdílný. Emise CO_2 jsou určovány převážně teplotou a většina pokusných komor měla shodný trend průběhu emisí. Naproti tomu emise N_2O byly nejenom velmi variabilní, ale také každá komora měla svůj specifický průběh emisí, proto vztah mezi emisemi N_2O a teplotou byl komplikovanější. V souladu s výsledky důrazně doporučujeme detailní výzkum včetně častého vzorkování v době vysokých plynných emisí, jako způsob jak přesněji stanovit celkové plynné emise v delších časových úsecích.

V

High fluxes but different patterns of N_2O and CO_2 emissions from soil in a cattle overwintering area

Hynšt, J., Šimek, M., Brůček, P., Petersen, S. O., 2007. High fluxes but different patterns of nitrous oxide and carbon dioxide emissions from soil in a cattle overwintering area. Agriculture, Ecosystem and Environment 120, 269-279.

Podíl na publikaci 20%

Abstract

Emissions of N_2O and CO_2 from a cattle overwintering area were measured during two years. The measurements were performed at three sampling locations along a gradient of animal impact (severe, moderate, slight) to test the hypothesis that emissions of CO_2 and N_2O are positively related to the degree of impact. In addition to CO_2 and N_2O fluxes, soil mineral nitrogen (NH_4^+ and NO_3^-), pH and temperature were determined to assess possible regulations of gas fluxes. Deposition of animal excreta resulted in a significant accumulation of nitrogen in the soil during winter. Most of the N_2O was emitted during a few short periods in spring and/or in late autumn. Large N_2O fluxes were associated with recent rainfall on some sampling dates. During winter and spring, presumably in periods of increasing temperatures, intensive soil nitrogen transformations took place, followed by peaks of N_2O emissions. Maximum N_2O fluxes of up to 2.5 mg N-N_2O m^{-2} h^{-1} were usually recorded at the most impacted location near the animal house, where also the highest concentrations of mineral nitrogen occurred. However, the effect of animal impact was not simple and on some occasions N_2O emissions were higher at the moderately impacted location. The emissions of CO_2 showed a completely different pattern than those of N_2O, being correlated with soil temperature; the highest emissions thus occurred in June-July, while very low fluxes were found in winter. Emission values ranged from about zero to 700 mg C-CO_2 m^{-2} h^{-1}. Further, the effect of animal impact on CO_2 emissions was opposite to that on N_2O fluxes, as the highest CO_2 fluxes were mostly recorded at the least impacted location, where likely respiration of plants increased overall CO_2 production. The potential for N_2O reduction to N_2 was determined in severely and moderately impacted soil in early autumn using acetylene inhibition; N_2 was generally the main nitrogen gas emitted. To test the relationship between rainfall and N_2O emissions, experimental wetting of dry soil was performed which caused a sharp, but short-lived increase of N_2O emissions.

Abstrakt
Zimoviště skotu mohou být významným zdrojem emisí významných skleníkových plynů, CO_2 a N_2O. Bylo provedeno dvouleté sledování emisí N_2O a CO_2 z půd zimoviště. Měření probíhalo na třech bodech podél gradientu vlivu zvířat s cílem ověřit hypotézu, že emise N_2O a CO_2 jsou úměrné vlivu zvířat. Byly také měřeny obsah minerálního dusíku, hodnota pH a teplota s cílem zjistit vliv regulačních faktorů na emise. Hromadění exkrementů zvířat na zimovišti silně zvýšilo obsah dusíku v půdě během zimních měsíců, ale většina N_2O vznikla během krátkých period na jaře a na podzim. Nejvyšší hodnoty emisí byly naměřeny na ploše nejvíce zatížené zvířaty poblíž kravína, kde byla také nejvyšší koncentrace dusíku v půdě. Emise CO_2 měly zcela odlišný vývoj než emise N_2O a byly přímo úměrné teplotě půdy s nejvyššími hodnotami v období červen – červenec. Z výsledků vyplývá, že zimoviště je významným zdrojem skleníkových plynů, včetně N_2O a CO_2. Nicméně emise obou plynů mají odlišný průběh během roku a zřejmě jsou regulovány odlišnými faktory prostředí.

VI

Effects of excretal returns and soil compaction on nitrous oxide emissions from a cattle overwintering area

Šimek, M., Brůček, P., Hynšt, J., Uhlířová, E., Petersen, S.O., 2006. Effects of excretal returns and soil compaction on nitrous oxide emissions from a cattle overwintering area. Agriculture, Ecosystem and Environment 112, 186-191.

Podíl na publikaci 40%

Abstract

Excretal returns and physical disturbance due to treading can greatly influence nitrogen flows in grazed pastures. Dung and urine depositions stimulate microbial transformations, while soil compaction and poaching change the physical environment in which these transformations take place. In this study, a cattle overwintering area in the Southwest Czech Republic was characterized with respect to bulk density, porosity, water-filled pore space (WFPS), organic C, total N, pH, microbial biomass C and denitrifying enzyme activity (DEA). Carbon dioxide and nitrous oxide (N_2O) emissions were measured on four different dates between October 2001 and May 2002. Sampling took place along a transect away from an open barn with access to feed. Soil chemical and biological properties showed that deposition of excreta declined with distance from the barn. In contrast, N_2O emissions were highest at intermediate positions along the transect. At the section with the greatest animal impact, the ratio of N_2 versus N_2O produced was five-fold higher, and the soil pH was 2 units higher, compared to the section with the least animal impact, which indicated that soil conditions favoured production of N_2 rather than N_2O in the area where excretal returns and treading was intense. A multiple linear regression was conducted using data from the last sampling. There were significant effects of WFPS and pH on log-transformed N_2O emissions, while effects of NH_4^+ and NO_3^-, and interactions between NH_4^+ and, respectively, WFPS and pH were nearly significant. The observations indicate that, whereas pasture management to achieve a better distribution of animal impact may improve N retention in the soil, it is not clear whether this will reduce N_2O emissions.

Abstrakt

Exkrementy zvířat a narušení povrchu vlivem pohybu pri pastvě silně ovlivňují mikrobní transformace dusíku v půdách pastvin. Emise oxidu dusného a oxidu uhličitého byly měřeny ve čtyřech termínech od října 2001 do května 2002. V půdě byla stanovena objemová hmotnost, pórovitost, podíl pórů vyplněných vodou (WFPS), organický C, celkový N, pH, uhlík v mikrobní biomase a aktivita denitrifikačních enzymů (DEA). Vzorky byly odebírány na transektu s rostoucí vzdáleností od kravína. Chemické a biologické vlastnosti půd prokázaly, že přísun živin ve forme exkrementů klesal podél transektu směrem od kravína. Naopak, emise N_2O byly největší ve střední části transektu. V části zimovište nejvíce zatížené dobytkem bylo pH vyšší o 2 jednotky a poměr N_2/N_2O byl pětkrát vyšší než v části s nejmenší zátěží. Pomocí mnohonásobné lineární regrese byl zjišten průkazný vliv WFPS a pH na emise N_2O, zatímco vliv NH_4^+ a NO_3^- byl neprůkazný. Z výsledku vyplývá, že změny obhospodařování zimovište, zaměrené na rovnoměrnejší zatížení zvířaty, nemusí být nutně spojeny s redukcí emisí N_2O.

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