Emissions of N_2O from pasture soils
Emise N_2O z půd pastvin

Jaroslav Hynšt

Ph.D. Thesis
Doktorská disertační práce

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Supervisor: prof. ing. Miloslav Šimek, CSc.
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Annotation:
Emissions of N_2O from pasture soils in a cattle overwintering area were investigated. The study was based on frequent field measurements of emissions at three locations along the gradient of animal impact and on field experiments focused on effect of nutrients addition on N_2O emissions.

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I declare hereby that I worked out this thesis on my own only with the use of the cited literature and other cited sources.
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Contents

Chapter 1

General introduction

The formation of gaseous metabolites

The exchange of gases between atmosphere and living organisms is one of the most important processes supporting life on the Earth. The atmosphere is both source and sink of two basic elements necessary for production of biomass – carbon and nitrogen. Carbon in the form of CO_2 is continually taken from the atmosphere by photosynthesis and returned by respiration. Nitrogen (N_2) is built in biomass by nitrogen fixation and returned into the atmosphere by denitrification. Very important part of gas exchange between atmosphere and living organisms is connected with transformations of nutrients in soil. During transformations of nitrogen, oxidized gaseous metabolites and by-products are released, especially nitric oxide (NO) and nitrous oxide (N_2O). Nitrous oxide belongs among so called greenhouse gases. The most important processes producing N_2O are denitrification and nitrification. Increasing inputs of nitrogen from agriculture and other human activities into the environment increase also transformations of nitrogen in soil including processes responsible for emissions of N_2O. Important source of N_2O emissions are intensively managed agricultural ecosystems where intensive fertilization is often accompanied by gaseous losses of nitrogen.

Inputs of nitrogen and its transformations in pasture ecosystem

Microbes, plants and animals consume nitrogen according to their actual demands. On the other hand, they excrete nitrogen, too. The principal basic source of nitrogen is the atmosphere and most nitrogen, fixed in living biomass, has its origin in atmospheric N_2. Biological cycling of nitrogen starts by its uptake from the atmosphere during N_2 fixation and at the end nitrogen is returned back into the atmosphere by denitrification, which helps to maintain the atmosphere in a steady state. But in real soil, transformations of nitrogen seldom run as a simple closed cycle, from N_2 fixed by biological fixation to N_2 produced by denitrification; instead other significant inputs and outputs of N occur. In the case of pastures, the main sources of soil N are mineral and organic fertilizers, fixation of N_2 and cattle excrements and urine. The extent of inputs and proportion of sources are dependent on intensity of pasture management. In intensively fertilized pastures most of N is supplemented in the form of fertilization. Organic or mineral fertilizers are applied in doses, corresponding to 0 – 400 kg N ha^{-1}, and the importance of N_2 fixation is lower. On the other hand, it is important source of N in extensive pasture ecosystems. Most of N in soil (approximately 98 – 99% of total soil N) is in organic form composed from mixture of polymerized humic substances, proteins, amino acids and nucleic acids. The source of these organic compounds are organic fertilizers, dung in various degree of decomposition, plant residues, dead microbial cells, urine, etc. Organic N is either conserved in soil as recalcitrant compounds (highly polymerized humic acids) or converted to NH_4^+ by mineralization. It is further lost from the soil as NH_3, oxidized to NO_3^- by nitrification or utilized by plants or microorganisms for production of biomass. Nitrate is consumed especially by plants as a source of N. Microbes usually prefer NH_4^+ form for biomass production, but they also use NO_3^- as source of nitrogen or oxidative agent instead of O_2, according to their actual demand. Soil mineral N usually creates 1 – 2% of total N and its content undergoes fast changes. The content of total N is relatively stable, changing slowly even at high rates of organic N inputs, as losses are proportional to inputs. Nitrogen in plant biomass is partly recycled by animals, their excrements and urine contributes to N inputs. Grazing on a mixed grass-clover pasture, animals retain 5 – 20% of N for maintenance and production and the rest is excreted as dung or urine. In urine, 70% of N is in the form of urea, while in dung most of N is in organic form created by microbial biomass (60 – 65%), soluble N (20 – 25%) and undigested N from the fodder (Oenema, 1997).

The consumption and the excretion of nitrogen by plants and soil microbes

Plants are usually considered as the driving component in ecosystems. They utilize especially mineral forms of nitrogen (NH_4^+, NO_3^-), their demands for nitrogen is a strong regulator of soil mineral nitrogen content and in conditions of limited nitrogen availability, competition on nitrogen between plants and microbes take place. Daily consumption of mineral nitrogen by plants is up to 5 kg ha^{-1} (Jackson et al., 1989; Ledgard et al., 1998). On the other hand, the plants also produce some N in root exudates. Soil microbes consume both mineral and organic forms of nitrogen. They can utilize mineral or organic nitrogen for production of biomass, and reduce oxidized forms, namely NO_3^--N during denitrification, but they also produce NH_4^+ -N out of organic forms during mineralization and NO_3^- -N out of NH_4^+ during nitrification (to mention just the most frequent nitrogen transformations). Consumption of N by microbes is in the range simillar to the demands of plants (Jackson et al., 1989; Ledgard et al., 1998). The relationship between consumption and production of nitrogen is regulated by the availability of other nutrients necessary for the assimilation of nitrogen by plants and microbes, especially carbon (except of nitrification). Pasture soils are usually rich in carbon, but input of N often highly exceeds the availability of carbon for nitrogen assimilation, the immobilization is exceeded by mineralization and as a result mineral forms of nitrogen accumulate in soil.

Processes producing N_2O

Nitrous oxide (N_2O) is produced in soil in various oxidation and reduction processes, namely during the oxidation of ammonium nitrogen (i. e. as a by-product in nitrification normally yielding nitrates: $NH_4^+ \rightarrow NO_3^-$) and during the reduction of nitrate nitrogen (i. e. as one of the two main gaseous products of denitrification: $NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$) (Skiba et al. 1992; Lovell, Jarvis 1996; Oenema et al. 1997; Bremner 1997). Nitrous oxide production during nitrification likely occurs in conditions of decreasing partial pressure of molecular oxygen in a close vicinity of active nitrifiers that normally utilize oxygen as a final electron acceptor in oxidation-reduction (=respiration) reactions. Under conditions of decreased availability of molecular oxygen and under other specific environmental conditions characterized by high ammonium-N content and low available C content the nitrifiers tend to use nitrite or even nitrate as a terminal electron acceptor; the process, that is called nitrifier denitrification, thus produces substantial amounts of nitrogen oxides, NO and N_2O (Wrage et al. 2001; McLain, Martens, 2004). Extreme level of nutrient input, usually observed in pasture soils, exceeds the capacity of the soil to utilise nitrogen without producing gas metabolites. The high amount of NH_4^+-N could stop the second reaction of nitrification (oxidation of NO_2^- to NO_3^-) causing temporary accumulation of NO_2^- (Oenema et al. 1997). Nitrite is toxic and microbes reduce it to various gas products (N_2, N_2O, NO) to detoxify their environment

(Bremner, 1997). N_2O can be also produced as by-product of both partial steps of nitrification - too intensive reaction or environmental conditions not suitable for nitrification (low pH value, low O_2).
Denitrification does mean the reduction of NO_3^- to N_2. For denitrification special conditions are necessary – low concentration of O_2 and sufficient amount of carbon and NO_3^-. In such conditions, microbes growing in soil start to respire NO_3^- instead of O_2. In pasture soils, conditions favourable for denitrification often take place. Carbon and nitrogen from excrements accumulate in large quantities and soil is compacted by cattle traffic. Nitrogen from dung and urine is readily converted to NO_3^- and it is available for respiration of carbon in microlocalities with restricted aeration. In addition intensive respiration consumes O_2 and alternative electron acceptors are necessary even in well aerated soil. The final product of denitrification in optimum conditions should be N_2, but, if the amount of available oxidized nitrogen (NO_3^-, NO_2^-) in environment exceeds the capacity of the N_2O reduction, significant amounts of N_2O are evolved and release the soil (Nevison, 1996).

Losses of nitrogen

The ability of soil to store nitrogen is limited and excessive inputs are usually connected with nitrogen losses. In terrestrial ecosystem, nitrogen is conserved in soil organic matter, in living plants or in biomass of microbes and animals. As plants are primary producers, the ability of other organisms to consume nitrogen is limited by their production of organic nitrogen compounds out of soil mineral nitrogen. Products of photosynthesis accumulate in plant tissues, and sugars, organic acids and other compounds are then used by microbes or animals for production of their own nitrogenous constituents of biomass. In ecosystems with low inputs of nutrients, nitrogen is efficiently reutilized and losses are low. If nitrogen input exceeds its living organisms requirement, it is lost in various forms. Nitrogen in the form of NH_4^+ (added in mineral fertilizers or produced during decomposition of organic matter) is often lost as NH_3 that escapes into the atmosphere. Oxidation of NH_4^+ produces NO_3^-. Its intensive production and accumulation is often accompanied with loses of various oxidized nitrogen compounds, as two important nitrogen oxides – NO and N_2O - are released during nitrification, too. Nitrate accumulated in soil in excess is lost from the soil environment by leaching or denitrification. Loses of nitrogen oxides are often mentioned to be proportional to inputs of nitrogen into the soil. Using the idea of Davidson (1993) of the soil N cycle, as "a flow through the leaky pipe", the "leakages" from the system seem to be positively correlated with the "flow rate". Emissions are often expressed as % of N-N_2O from nitrogen input. For example Velthof et al. (1997) found losses of N-N_2O within the range 0.1 - 8% of nitrogen applied as organic or mineral fertiliser (cattle slurry, calcium ammonium nitrate, ammonium sulphate). Fowler et al. (1997) in their revue reported values 1.2 - 6.7 % of fertiliser and excreta nitrogen lost as N_2O at the grazed grassland. According to extensive analysis of emission data from various fertilized and unfertilized agricultural soils and grasslands, Bouwman (1996) reported equation for calculation of N_2O emissions in agricultural soils: $E = 1 + 0.0125\ F$, where E represents mean annual emission of N_2O (kg N ha^{-1} y^{-1}) and F is amount of applied fertilizer (kg N ha^{-1}).

Aims and research questions

General hypothesis: Pastures are important source of N_2O because cattle traffic and dung deposition create conditions favourable for N_2O emissions from soil. Accumulation of nutrients increases microbial activity and supports intensive transformations of nitrogen which are accompanied by production of N_2O.

We hypothesized that effects usually observed in pasture ecosystems are more profound at overwintering area, where cattle spend winter time and therefore overwintering area is important source of N_2O. Presence of high number of animals on small area during winter cause extreme accumulation of nutrients. Nutrients accumulated in soil undergo intensive transformations and also processes producing N_2O are very intensive.

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.

We also hypothesized that emissions of N_2O increase with increasing animal impact along this gradient. Two year measurements of emissions were performed aiming to estimate annual emissions of N_2O at three differently impacted soils along the gradient (chapters 2 and 3). Ratio of denitrification products N_2 and N_2O at three locations along the gradient was determined in experiment using ^{15}N (chapter 4). The emissions of N_2O are extremely heterogeneous in space and time and therefore we performed experiments with artificial application of different amounts of nutrients (chapters 5 and 6). The aim of experiments was to determine relationship between inputs of nitrogen and emissions of N_2O.

Outline of the thesis

Chapter 1 represents a review of soil nitrogen cycle and processes responsible for production and emissions of N_2O. There are also formulated hypothesis and aims.

Chapters 2 and 3 contain results of field measurements of N_2O emissions. Emissions were measured at three locations of overwintering area with different animal impact. According to results, annual emissions of N_2O from overwintering area were estimated. Effects of selected environmental variables on emissions were also determined.

Chapter 4 describes results of field determination of ratio N_2/N_2O along the gradient of animal impact. The production and ratio of denitrification products were estimated using application of ^{15}N-labeled nitrate.

Chapter 5 contains results of field measurements of N_2O emissions after application of high doses of glucose and nitrate. The aim of experiment was to determine how much N_2O could be emitted from the soil saturated by nitrogen.

In chapter 6, field experiment with addition of low amount of nitrate nitrogen into soil is described. The effect of nitrogen was compared in two soils with different impact of cattle.

In chapter 7, the most important results are summarized and discussed and concluding remarks are defined.

Chapter 2

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 10%

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.

Chapter 3

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 50%

Abstract

Cattle overwintering areas common in central Europe may represent significant point sources of important greenhouse gases. A field study was carried out during two years to estimate the emissions of N_2O and CO_2 from soil in a cattle overwintering area located in the southwest Czech Republic. 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 determined by using non-vented manual closed chambers, soil mineral nitrogen (NH_4^+ and NO_3^-), pH and temperature were determined to assess their regulatory role and impact on gas fluxes. The overwintering area was about 4 ha and it had been used for overwintering of about 90 cows since 1995. Deposition of animal excreta resulted in a significant accumulation of nitrogen in the soil during winter, but 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, characterized by a diminishment of previously accumulated NH_4^+-N and by changes in NO_3^--N content, 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 recorded at the most impacted location near the animal house, where the highest concentrations of soil mineral nitrogen also 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 to 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}. Furthermore, 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 respiration of plants most likely increased overall CO_2 production. The results show that cattle overwintering areas are important sources of greenhouse gases, including N_2O and CO_2. Fluxes of these two gases are, however, differently distributed over the year, which also suggests that they are controlled by different environmental and soil factors.

Chapter 4

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. 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, 2006, 343-346.

Podíl na publikaci 10%

Abstract

The grassland area where cattle overwinter was identified as an important point source of N_2O due to soil compaction and excrement deposition. The rates of N_2O emission 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.

Chapter 5

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

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

Podíl na publikaci 50%

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.

Chapter 6

The effect of small amounts of nitrate on N_2O emissions in two different soils

Hynšt, J., Šimek, M., Brůček, P. The effect of small amounts of nitrate nitrogen on N_2O emissions at two soils at overwintering area with different animal impact. In preparation.

Podíl na publikaci 50%

Abstract

The objective of this study was to examine short term effect of nitrogen addition into soil on emissions of N_2O in two soils of overwintering area differently affected by cattle. The addition of nutrients increase the amount of NO_3^--N in soil. The effect of application of increasing amount of fertilizer on NO_3^--N in soil is more profound in disturbed soil than in undisturbed soil. Presence of NO_2^- in soil at the location M without any amendement suggest some disturbance of nitrogen cycle. Emissions of N_2O are proportional to increase of NO_3^- and NO_2^- after fertilization and in result cumulative production of N_2O is higher in disturbed soil at the location M, but in some ocassions, emissions of N_2O in undisturbed soil at the location C were lower at soil nitrate content exceeding this one at the location M. Possible reasons for such effect are discussed. Emissions of N_2O increase rapidly after amendement, but the effect is short-term and emissions usually decreased at still high soil nitrate content. The amount of nitrogen which does not increase cumulative production of N_2O is questionable. More probably, the increase of soil nitrogen content always increase also the emissions of N_2O, but this effect could be too low and insignificant.

Chapter 7

General discussion

The cattle overwintering area is an ecosystem where high inputs of nutrients create conditions favourable for intensive microbial activity including processes of nitrogen transformations. Nutrients in the form of dung and urine deposition accumulate during winter time, when high number of animals (90 cows in the farm under study) is present on relatively small area (4 ha). Part of carbon and nitrogen is retained in soil as plant and microbial biomass and soil organic matter, but their long-term build up is relatively weak in comparison with deposition, which suggests significant losses of nutrients. Organic nitrogen from cattle excrements is rapidly converted to N-NH_4^+ that temporarily accumulates in soil in high concentrations; it is further oxidized to N-NO_3^- in early spring or even during warm periods in winter, when processes producing N_2O, especially nitrification and denitrification, take place and also most of N_2O is emitted. Changes of soil mineral nitrogen content were rapid, which suggests very intensive transformations of nitrogen. This assumption is in agreement with observed values of emissions of N_2O. Emissions as high as 3 000 – 8 000 ug N-N_2O m^{-2} h^{-1} determined at the overwintering area represent one of the highest values reported in field conditions and only high "flow through the pipe" (Davidson, 1993) should create conditions for such production of nitrogen gases. Emissions of N_2O usually increased with increasing dung accumulation and destruction of soil surface in agreement with the rate of cattle impact along the gradient, although on some occasions, N_2O production from less impacted parts of the overwintering area exceeded that from the most impacted locations. This is likely a result of enhanced reduction of N_2O to N_2, thus lowering the amounts of N_2O emitted. Annual production of N-N_2O estimated during two years of relatively frequent measurements was 1 – 15 kg N ha^{-1}, according to the intensity of cattle impact. Large potential of soil for production of N_2O by denitrification was also observed during field experiments.

Increasing doses of nutrients increased cumulative values of emissions proportionaly to the level of fertilization without any limit in a broad range of nitrate-nitrogen inputs (0 – 1 500 kg N ha^{-1}). On the other hand, even the lowest inputs of nitrogen into soil (5 kg N ha^{-1}). increased emissions of N_2O. The experimentaly 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 agreement with previously reported values (Velthof, 1997; Fowler 1997; Lampe et al., 2006; McTagart et al., 1997). According to results of our experiments, possible range of cumulative annual emissions was estimated. Nitrogen input was calculated, on the base of data provided by the farmer, to 180 kg N ha^{-1}. If we assume emission factors 0.2 – 5.6% of nitrogen input established in our experiments, soil is able to produce 0.4 – 10.0 kg N-N_2O ha^{-1}, which is in agreement with the range of losses 1.3 – 12.6 kg N ha^{-1}, observed in field conditions during the period October – May, when most of N_2O is usually emitted.

Increase of N_2O emissions after nitrogen amendment to soil with low animal impact was usually lower in comparison with more affected one. Higher emissions of N_2O at the location with higher effect of cattle are probably not only stimulated by higher content of nitrogen, but they are also conected with higher microbial activity. The less animals meant the lower inputs of nutrients and microbes competed with plants on nitrogen. In result, microbes were less active and microbial comunity needed less nitrogen for denitrification.

Our results and other data reported on nitrogen cycling in ecosystems also suggest some mitigation options of N_2O emissions. Basic possibility how to decrease emissions of N_2O is decreasing the nitrogen input. The nitrogen input could be decreased by decreasing of the animal density on the pasture area or by decreasing of nitrogen excretion of individual animals. The amount of nitrogen released by animals is strongly affected by the composition of the diet. When dietary protein is in excess of the amount required for ruminal microorganisms, the protein is degraded to ammonia N, absorbed, metabolized to urea in the liver and lost in the urine (Bach et al., 2005). Manipulating the diet of animals could decrease the loss of nitrogen in urea and, in result, decrease nitrogen available for processes producing N_2O in soil. Reduction of emissions by reducing nitrogen excretion of pigs was reported by Chadwick (1997). Decreasing protein content of pig diet resulted in lower nitrogen content in slurry applied to grassland soil and significantly lowered emissions of NH_3 and N_2O.
In the case of overwintering area, decreasing the number of animals and manipulating the diet have a limited value for mitigation of N_2O emissions. Emissions are naturally lowered in some extent by reduction of N_2O to N_2, which is higher at locations with higher impact of animals. Possible explanation of this effect is a higher requirement for oxidation agents in soil where microbial activity is stimulated by higher content of carbon and other nutrients from cattle excrements. In addition, increased soil pH, observed in our studies at locations with higher activity of grazing, has been reported to stimulate reduction of N_2O to N_2. It suggests increasing density of animals as a possible theoretical way, how to decrease emissions of N_2O. However, increased density of cattle would increase the input of nitrogen into the soil. Application of organic matter with low content of nitrogen and high content of carbon sources (for example straw, glucose) into the soil should increase the consumption of nitrogen by processes different from denitrification, but real effect of this treatment and especially its practical applicability is questionable.

In general, possibilities of mitigation of emissions at overwintering areas are only limited and these areas will be probably always the significant source of N_2O.

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Šimek, M., Elhottová, D., Uhlířová, E., Šantrůčková, H., Brůček, P., **Hynšt, J**., Kamír, V.: Soil microbial community in pasture soil: impact of cattle traffic and excretal returns. In: Structure and function of soil microbiota. International Symposium. Marburg, September 18-20, 2003, p. 167.

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