



How shallow water table conditions affect N₂O emissions and associated microbial abundances under different nitrogen fertilisations

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ABSTRACT

Globally, agriculture is the largest source of nitrous oxide (N₂O), a potent greenhouse gas (GHG). A recognised tool to prevent its loss from agricultural soils is the presence of a shallow water table. A four-year lysimeter experiment (2011–2014) was conducted in northeast Italy to investigate how water table levels affect N₂O emissions under different N fertilisation techniques. Soil surface flux and groundwater-dissolved N₂O were studied under free drainage and at two shallow water table levels (60 cm and 120 cm) and at two levels of N input (250 and 368 kg N ha⁻¹ y⁻¹), using dry manure in 2011 and 2012 and fresh manure in 2013 and 2014. DNA was extracted from soils and quantitative PCR (qPCR) was used to assess the size of nitrifying, denitrifying and N₂-fixing bacterial communities, at three soil depths. The day after pre-seeding fertiliser incorporation, N₂O emission started to be detected and continued for two-three weeks; brief measurable emissions also followed top-dressing fertilisation events. Cumulative N₂O emission measured between 0.97 and 2.33 kg N₂O-N ha⁻¹ y⁻¹, corresponding to emission factors from 0.4% to 1.1%. Manure fertilisation significantly affected the N dose only when applied as fresh manure. Water-filled pore space (WFPS) affected daily N₂O emissions with a significant interaction with fertilisation level. The two N input levels showed differences only when WFPS was > 40%, which revealed N availability as key to increased N₂O emissions at high water content, supposedly by fostering anaerobic denitrification. No significant relationships were observed between peak N₂O emissions and the values of the temperature or irrigation variables recorded during the experimental observation period. Groundwater dissolved N₂O-N concentrations measured about 1.7 μg L⁻¹ with some peak variability from nitrate leaching. Quantitative PCR assays demonstrated that shifts in microbial population that can be involved in oxidation processes and heterotrophic denitrification occurred in the soil, even though the contributions of the different N pathways on N₂O emissions were indistinguishable. Indeed, both nitrifying and denitrifying genes were simultaneously promoted by the high fertilisation input and hindered by the high water table level. Shallow groundwater conditions appeared to reduce N₂O emissions probably by favouring complete denitrification. These results suggest that in the Po Plain, regulated by the Nitrate Directive, shallow groundwater conditions, with a balanced N input, may mitigate air and water pollution.

1. Introduction

Intensive agriculture applies nitrogen (N) at levels required to optimise crop productivity. However, an unbalanced N input increases the potential risk of groundwater contamination from nitrate leaching (Spalding and Exner, 1991; De Simone and Howes, 1998; Liao et al., 2012), of ammonia volatilisation (Lourenço et al., 2016), and of atmospheric contamination from nitrous oxide (N₂O) emissions

(Bouwman, 1990, 1996; Isermann, 1994) rises.

Agriculture is the largest source of N₂O, which has long been considered a major anthropogenic greenhouse gas. Nevertheless, our understanding of N₂O production — pivotal for identifying mitigation options — is relatively limited due to the complexity of the N biogeochemical cycle in soil.

Nitrification and denitrification are known to be the main pathways of N₂O production and are controlled by enzymatic activities of soil

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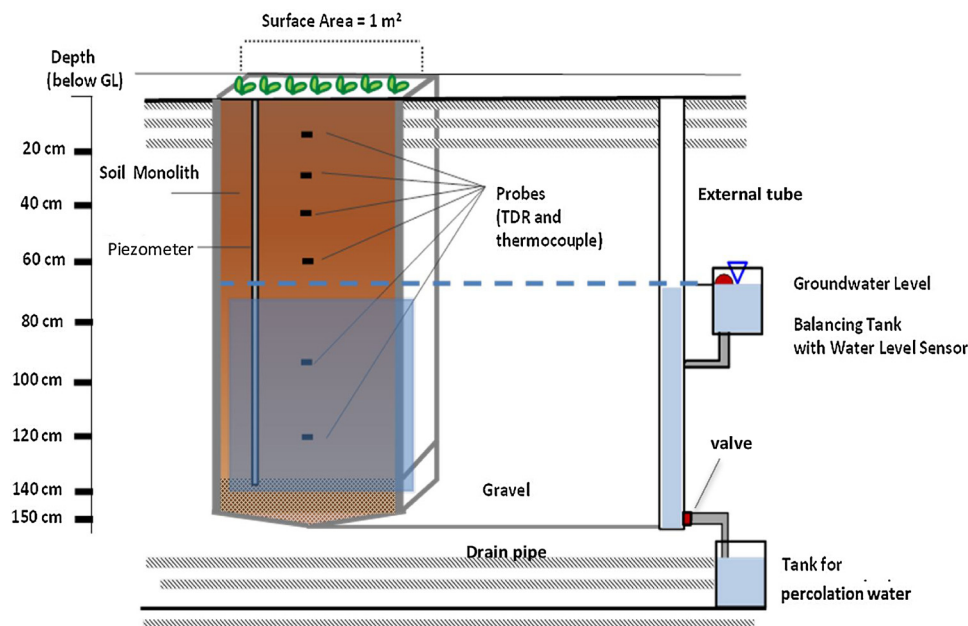


Fig. 1. Lysimeter cross-section.

microbes. The two factors that most affect N_2O emission are the genetic capability of microorganisms to perform the steps of the two pathways and the environmental conditions required for expression of such genetic potential (Saggar et al., 2013; Morales et al., 2015). The latter factor includes NO_3^- and NH_4^+ supply, C availability (electron donors), temperature, soil redox conditions linked to water filled pore space (WFPS%), consequent aeration status, and pH (Hansen et al., 2014; Samad et al., 2016).

Denitrifying bacteria are ubiquitous in agricultural soils (Payne, 1981) and tend to concentrate around soil matrix microsites, where oxidisable organic matter is highly available and/or the conditions limit oxygen diffusion. Non-limiting nitrate concentrations are also required. The lack of any one of these conditions may block denitrification at the N_2O step (Firestone, 1982; Firestone and Davidson, 1989; Robertson and Groffman, 2007).

Nitrous oxide production is linked most often to heterotrophic denitrifiers, however it is not restricted to those pathways. Nitrifiers can also produce it under certain conditions, such as when WFPS does not exceed 60% (i.e., when oxygen becomes limiting due to its low solubility and consequent slow diffusion in water) (Bateman and Baggs, 2005). Indeed, nitrifiers can reduce NO_2^- to N_2O or N_2 under short-term O_2 limitation (“nitrifier-denitrification”) (Foth and Ellis, 1996), but nitrification ceases in the absence of O_2 .

Since optimal conditions for complete denitrification of N_2 are generally found when O_2 supply is limited, the presence of a shallow water table (providing an anaerobic environment) is recognised as a potential factor for preventing N_2O losses from soils (Flessa et al., 1998; von Arnold et al., 2005).

Nitrous oxide can also be produced within groundwater. Following its dissolution in subsurface drainage and groundwater, N_2O is emitted to the atmosphere through open surface degassing and upward flux diffusion (Minamikawa et al., 2013). Its high solubility and slow diffusion permit it to remain in shallow groundwater for extended periods after production. Under such conditions, N_2O is far likelier to be re-consumed and recycled by either denitrifiers or other microbes, thereby lowering emissions (Skiba et al., 1997).

Morari et al. (2012) showed in a recent study performed in northeast Italy that areas with shallow groundwater seem less vulnerable to N pollution relative to the predictions from the index-based model DRASTIC (Rundquist et al., 1991) for NVZ (nitrate vulnerable zones)

assignment. The authors found, in fact, low N concentrations in soils fertilised with manure in the presence of a shallow water table. The water table level appeared to influence the recovery of N leached in the root zone by upward water movement. These findings put forth that limited O_2 supply may significantly affect N gaseous losses (N_2O and N_2 emissions). From an environmental perspective, a $N_2O:N_2$ ratio shift in favour of N_2O would reduce any water quality benefit obtained from denitrification (Elmi et al., 2005). Conversely, highly anoxic conditions caused by a shallow water table could favour N_2O consumption and reduce overall N_2O emissions (Hansen et al., 2014).

Based on this evidence, a four-year lysimeter experiment was set up to evaluate the effect of shallow water table conditions on N_2O emissions and the consistency of the different microbial populations involved in N cycling pathways. We hypothesised that shallow groundwater could limit nitrification in favour of denitrification, and in turn mitigate N_2O fluxes from soil surface. Although these hypotheses are not directly testable by enumerating bacterial gene abundances, their upshifts are nevertheless interpreted as reflecting the product of the active physiology underlying the possess of the genes analysed.

2. Material and methods

2.1. Site description

The study was conducted at the experimental farm of the University of Padua, in northeast Italy (45°19'N, 11°31' E, 8 m a.s.l.) from May 2010 to December 2013. The local sub-humid climate has an annual average temperature and rainfall of 12 °C and 800–850 mm, respectively. Annual rainfall is mostly concentrated during the autumn and spring months. Reference evapotranspiration (ET_0) is 800 mm and peaks in July (4.5 mm d^{-1}) (Veneto Region Environmental Protection Agency, ARPAV).

The study site was originally set up in 1984 and consisted of twenty 1 m × 1 m × 1.5 m (length × width × depth) drainable lysimeters. The bottom of each lysimeter is funnel-shaped and connected via an underground drain-pipe (1‰ slope) to an external tube, fitted with a valve that regulates both the water table level and leaching discharge (Fig. 1).

Each lysimeter was filled in 1984 with soil excavated from the adjacent experimental farm, in a way that preserved the original soil horizons. To facilitate water drainage and prevent soil washout, a

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