Soil Biology & Biochemistry 84 (2015) 96–106

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Heterogeneity of O_2 dynamics in soil amended with animal manure and implications for greenhouse gas emissions

Kun Zhu [a], Sander Bruun [a], Morten Larsen [b], Ronnie N. Glud [b], Lars Stoumann Jensen [a, *]

[a] *Department of Plant and Environmental Sciences, University of Copenhagen, Denmark*
[b] *Nordic Center for Earth Evolution, Institute of Biology, University of Southern Denmark, Denmark*

ARTICLE INFO

Article history:
Received 7 August 2014
Received in revised form
2 February 2015
Accepted 4 February 2015
Available online 21 February 2015

Keywords:
Oxygen optode
Oxygen dynamics
Manure distribution
Nitrification
Nitrous oxide emission

ABSTRACT

Soil oxygen (O_2) availability influences nitrification and denitrification, the major biological processes responsible for nitrous oxide (N_2O) production and emissions from soil. In this study O_2-specific planar optodes were used to visualise O_2 distribution with high spatial and temporal resolution in soils in which the same amount of solid fraction of pig manure had been distributed in three different ways (mixed, layered, single patch) and which were maintained at a water potential of −5 kPa (corresponding to 91% of water-filled pore space). In parallel, the greenhouse gas emissions (N_2O, CO_2 and CH_4) from soil at high temporal resolution were monitored. At the end of the incubations, vertical profiles of mineral nitrogen (ammonium and nitrate) in the soil matrix were quantified. The optode results revealed that anoxia rapidly developed in zones with manure addition and gradually expanded to the entire soil during the 66-h experimental period. The anoxia in the soil developed more quickly as the heterogeneity of manure distribution decreased (from single patch to layered to mixed). The single patch distribution of manure solids delayed peak emission rates of both N_2O and CO_2, but stimulated the cumulative N_2O emissions and reduced the cumulative CO_2 fluxes. The faster the anoxia developed, the less the nitrification process appeared to contribute to N_2O emissions. No treatment effects on CH_4 emissions were observed. Combined high resolution imaging of O_2 dynamics and measurements of N_2O emission rates are essential to get a detailed understanding of how O_2 availability regulates the distribution and coupling of denitrification and nitrification activity in soil. Such unique information on soil O_2 dynamics could be used for further modelling and quantification of processes producing greenhouse gases from soil ecosystems.

1. Introduction

Emission of greenhouse gases from soil (particularly N_2O) is controlled by complex biogeochemical processes. Soil O_2 availability is one of the main regulating factors, as O_2 controls denitrification through the activity and synthesis of denitrifying enzymes in soil (Firestone et al., 1980; Parkin et al., 1984). Denitrification is traditionally considered to be an anaerobic process (Smith, 1980). However, many soil denitrifying micro-organisms are apparently able to produce N_2O in micro-oxic environments (Robertson et al., 1989; Laughlin and Stevens, 2002). Conversely, nitrification is a strictly aerobic process since the NH_4^+ oxidation enzyme of nitrifying organisms requires O_2 for activation (Wood, 1986). The mechanisms of N_2O production by denitrification and nitrification are not completely elucidated, and in natural heterogeneous and dynamic soils, the regulation of N_2O turn-over is highly complex (Butterbach-Bahl et al., 2013). For instance, the application of fertilisers to agricultural soils will induce an extensive spatial variability in the distribution of water and substrates for microbial growth. Animal manure, commonly used as organic fertiliser contains substantial quantities of microbially available carbon (C), mineral nitrogen (N) and water, thereby provides the essential substrates for the microbial production of N_2O through nitrification and denitrification (Butterbach-Bahl and Dannenmann, 2011). Amended patches of manure provide 'hot spots' for microbial activity, and the combination of a high water content and intensified microbial oxygen (O_2) consumption will induce extensive small-scale variability in soil O_2 distribution. This may locally induce strong O_2 depletion and facilitate significant production of N_2O (Petersen et al., 1996). Besides respiration of organic matter, nitrification of ammonium also consumes O_2 and may further contribute to the O_2 depletion (Norton and Stark, 2011). Since denitrification in soil is dependent on the primary

---

* Corresponding author.
E-mail address: lsj@plen.ku.dk (L.S. Jensen).

http://dx.doi.org/10.1016/j.soilbio.2015.02.012
0038-0717/

products of nitrification – nitrite and nitrate (NO_2^- and NO_3^-) – the two processes are essentially linked. However, aerobic conditions are not optimal for denitrification and anaerobic conditions inhibit nitrification. Thus the co-occurrence of the two processes requires temporal or spatial differences in O_2 availability. In addition, manure application may possibly also stimulate emissions of carbon dioxide (CO_2) and methane (CH_4) (Heller et al., 2010; Montes et al., 2013).

When applied under natural conditions, manures and other organic amendments will commonly be heterogeneously rather than homogenously distributed in the soil matrix. This could affect C and N turnover processes as well as emissions of greenhouse gases (Wulf et al., 2002). Several studies have revealed the importance of spatial and temporal heterogeneity in soil O_2 concentrations for N_2O emissions (Meyer et al., 2002; Khalil et al., 2004; Morley and Baggs, 2010). Loecke and Robertson (2009) found that aggregation of plant litter slowed its decomposition and promoted N_2O emissions. They suggested that the reduced diffusion of O_2 played an important role for the reduced decomposition of litter aggregation in soil. Specifically, O_2 diffusion may match O_2 consumption more closely when litter is evenly distributed than when it is aggregated. Although considered as a key influencing factor, the soil O_2 concentrations have rarely been resolved at high spatio-temporal scales. The intense microbial activity associated with manure-amended zones induces steep concentration gradients of O_2, NO_2^- and NO_3^- that have previously only been resolved and investigated by microsensor approaches (Meyer et al., 2002, Markfoged et al., 2011). However, these kinds of sensors can only follow the dynamics in a few spots or through one-dimensional vertical profiling that only poorly approximate the true complexity of natural soils. Furthermore, micro-sensors are invasive, and a potential bias may arise from altered gas diffusivity around the sensor or after the sensor has been withdrawn. More detailed and non-intrusive measurements of soil O_2 distribution at high spatio-temporal resolution are required to identify and quantify the contributions of denitrification and nitrification to N_2O emissions.

Planar optodes have proven to be a versatile tool for imaging O_2, pH and pCO_2 distribution in sediments in particular (Frederiksen and Glud, 2006; Stahl et al., 2006; Zhu and Aller, 2010), and they have recently been introduced to soil science (Blossfeld and Gansert, 2007; Elberling et al., 2011; Zhu et al., 2014). Two dimensional distribution of O_2 level in soil can be monitored non/minimal-invasively with planar optodes. Those studies have underlined the great potential of planar optodes for understanding and investigating solute dynamics in natural complex soils with greenhouse gas emissions.

This study investigated the effects of the application modes of an organic amendment (pig manure, containing labile carbon and ammonium) and mineral ammonium on N_2O, CO_2 and CH_4 emissions in agricultural soil. By varying the initial spatial distribution of manure and mineral ammonium levels in the soil, the objective was to address the following questions: (1) Do the modes of manure distribution and addition of mineral ammonium affect spatial and temporal O_2 distribution in soil? (2) Is the induced O_2 dynamic linked to N_2O, CO_2 and CH_4 emissions? and (3) Do the modes of manure distribution influence the importance of nitrification for N_2O emissions?

2. Materials and methods

2.1. Preparation of soil and manure

Soil was collected on the experimental farm of the University of Copenhagen at Taastrup in Denmark (55° 40′ N, 12° 17′ E). The soil type is sandy clay loam (clay 15%, silt 18%, sand 65% and organic matter 2%) with 1.15% C, 0.13% total N, C/N of 9, soil pH (0.01 M $CaCl_2$) of 5.6 and cation exchange capacity of 8.4 cmol kg^{-1} at pH 7. The soil was re-moistened to 15.8% of gravimetrical water content and passed moist through a 2-mm sieve. The sieved soil was pre-incubated in a loosely closed box at room temperature (20 ± 3 °C) for two weeks prior to the experiment in order to allow the initial flush of respiration from newly-wetted soil to level off (Fierer and Schimel, 2003).

The solid fraction of manure was obtained by separating pig slurry using a screw press (Hjorth et al., 2010). Subsequently the solid fraction was stored at −18 °C until use. The manure solids consisted of 23.48% dry matter, 9.20% ash, 40.67 total C, 18.78 g kg^{-1} total N, 11.50 g kg^{-1} NH_4^+–N and 0.04 g kg^{-1} NO_3^-–N.

2.2. Experimental design

The experimental mesocosms consisted of six transparent boxes (height × length × width: 100 × 60 × 40 mm), each with a glass window insert (50 × 50 mm) on the front of the box equipped with an O_2 sensitive planar optode (see "Planar optode" section below). The optode box was repacked with 144 g (DM) soil by vertically shaking it until a bulk density of 1.5 g cm^{-3} was reached with a 4 cm depth of soil. The headspace in the box was 144 mL. The experimental design contained two variables: manure distribution and mineral NH_4^+–N addition. All boxes with manure addition received the same total amount of manure, but the way in which it was distributed differed. The distribution methods were as a single patch (PATCH), with manure placed in a cylinder shape and perpendicular to the front of the soil container, as a layer (LAYER) in the middle of the soil, or homogeneously distributed (MIX) with the manure well-mixed in the soil. There was also one control (CON) without any manure addition.

For each of the distributions and the control, there were treatments with (+) or without mineral NH_4^+–N. The amount of manure solids applied was equivalent to 4.3 g DM kg^{-1} soil, corresponding to 50 mg NH_4^+–Nkg^{-1} soil. The NH_4^+–N was added by mixing ammonium sulphate solution equivalent to 50 mg NH_4^+–Nkg^{-1} prior to amending the manure in the soil. For (MIX) treatments, manure solids were mixed thoroughly with the soil before compacting. LAYER treatments were made by compacting half of the soil (2 cm), placing the manure solids in a layer, and compacting the remaining half of the soil (2 cm) on top. PATCH treatments were made by compacting half of the soil, positioning the manure solids in a line perpendicular to the optode window, and compacting the remaining half of the soil on top (Fig. 1). In order to reach homogeneous soil density, the soil was compacted in all cases by shaking the optode box vertically. After adding the manure, the soils were adjusted to the designated water content by gently applying deionised water to the top of the soil using a pipette to avoid air bubbles being trapped at the bottom of the soil. Soil water potential was set to pF 1.7 (−5 kPa, 91% of water-filled pore space), and the corresponding gravimetric water contents were 27.4%. The six optode boxes (Fig. 2) were kept in the dark at 12 °C (±1 °C). The soil water contents were regularly checked and maintained by the addition of deionised water to the desired levels as required. Four trials were conducted sequentially: CON, PATCH, LAYER and MIX. In each trial there were three replicates for one treatment without mineral ammonium addition, and three other replicates for one treatment with mineral ammonium addition.

2.3. Planar optode

The specific applied imaging system and general measuring principle of planar O_2 optodes has previously been described in