Soil Biology & Biochemistry 57 (2013) 292–300

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

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

Soil environmental factors rather than denitrification gene abundance control N_2O fluxes in a wet sclerophyll forest with different burning frequency

Xian Liu [a], C.R. Chen [a,*], W.J. Wang [b], J.M. Hughes [c], Tom Lewis [d], E.Q. Hou [a], Jupei Shen [a]

[a] Environmental Futures Centre and Griffith School of Environment, Griffith University, Nathan, QLD 4111, Australia
[b] Soil Processes, Department of Science, Information Technology, Innovation and the Arts, 41 Boggo Road, Dutton Park, QLD 4102, Australia
[c] Australian River Institute and Griffith School of Environment, Griffith University, Nathan, QLD 4111, Australia
[d] Horticulture and Forestry Science Agri-Science Queensland, DAFF (Department of Agriculture, Fishery and Forestry), 4558, Australia

ARTICLE INFO

Article history:
Received 24 July 2012
Received in revised form
23 September 2012
Accepted 6 October 2012
Available online 2 November 2012

Keywords:
Prescribed burning
In situ N_2O flux
Soil environmental factors
Denitrification genes
Real-time PCR

ABSTRACT

Production of nitrous oxide (N_2O) by anaerobic denitrification is one of the most important processes in the global nitrogen (N) cycle and has attracted recent attention due to its significant impacts on climatic change. Fire is a key driver of many ecosystem processes, however, how fire drives the shift in microbial community and thus alters nutrient cycling is still unclear. In this study, a 35-year-old repeated prescribed burning trial, with three treatments (no burning, 2 yearly burning and 4 yearly burning), was used to explore how the long-term repeated prescribed burning affects N_2O flux, key soil properties (inorganic N, dissolved organic carbon (DOC) and N, pH, electrical conductivity (EC), moisture), denitrification gene abundance and their interactions. Soil samples were collected in January and April 2011. Quantitative real-time PCR was employed to quantify the gene copy number of target genes, including *nar*G, *nir*K, *nir*S and *nos*Z. *In situ* N_2O fluxes ranged from 0 to 8.8 g N_2O–N ha^{-1} h^{-1} with an average of 1.47 g N_2O–N ha^{-1} h^{-1}. More frequent fire (2 yearly burning) significantly reduced soil N_2O fluxes, availability of C and N substrates and moisture, but increased soil pH and EC compared with no burning and 4 yearly burning treatments. Fire treatments did not significantly affect the abundance of most denitrification genes. There were no significant differences in most parameters measured between the 4 yearly burning and no burning treatments, indicating microbial community function is not affected by less frequent (4 year interval) burning. Variation in the N_2O fluxes among the treatments can largely be explained by soil substrate (NO_3^-, DOC and total soluble nitrogen (TSN)) availability and soil environmental factors (pH, EC, and moisture), while the abundance of most denitrification genes were not related to the N_2O fluxes. It is concluded that soil environmental factors rather than denitrification gene abundance control N_2O fluxes in this wet sclerophyll forest in response to long-term repeated fires.

1. Introduction

Production of nitrous oxide (N_2O) by anaerobic denitrification is one of the most important processes in the global nitrogen (N) cycle and has attracted recent attention due to its significant impacts on climatic change (IPCC, 2007). This nitrogen (N) transformation process is of global interest because it can result in significant N losses from ecosystems, and the gaseous product N_2O depletes stratospheric ozone and contributes to global warming (Ravishankara et al., 2009). The N_2O is a potent greenhouse gas which has a global warming potential about 298 times greater than that of carbon (C) dioxide (Nakicenovic and Swart, 2000).

There is a large diversity of bacteria, archaea, and fungi involved in denitrification, and their abundance and composition are likely to be affected by changes in soil type and environmental factors. It is well known that soil organic C and NO_3^- availability (Klemedtsson et al., 2005), pH (Šlmek and Cooper, 2002), O_2 and water content (Bateman and Baggs, 2005) can influence the denitrification rates. These factors are likely to shape the variable structure and function of the microbial communities, whose composition mirrors and integrates the long-term effects of environmental change and resource availability (Wallenstein et al., 2006). On the other hand, the denitrifying community directly mediates the reducing processes of NO_3^- to produce N_2O and N_2. However, the processes/mechanisms governing the interaction of the denitrifying community, resource availability and environmental factors are largely unknown. Despite a large body of work carried out to explore the relationship between environmental

* Corresponding author. Tel.: +61 7 3735 7494; fax: +61 7 3735 7773.
E-mail address: c.chen@griffith.edu.au (C.R. Chen).

0038-0717/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.soilbio.2012.10.009

factors (soil pH, moisture, temperature, N substrate availability etc.) and denitrification (Weier et al., 1993; Bossio et al., 1998), fewer studies have attempted to gain a better understanding of the biological aspects of denitrification (Mergel et al., 2001; Rösch et al., 2002; Taroncher-Oldenburg et al., 2003; Wakelin et al., 2007).

Denitrification consists of four enzymatically catalyzed reductive steps: NO_3^- reduction ($NO_3^- \rightarrow NO_2^-$), NO_2^- reduction ($NO_2^- \rightarrow NO$), NO reduction ($NO \rightarrow N_2O$) and N_2O reduction ($N_2O \rightarrow N_2$) (Philippot, 2002; Ellen et al., 2006). These processes involve multiple genes encoding four metalloenzymes, including dissimilatory NO_3^- reductase, NO_2^- reductase, NO reductase, and N_2O reductase (Canfield et al., 2010). The dissimilatory NO_3^- reductase comprises two homologous enzymes- membrane-bound (Nar) and periplasmic-bound (Nap) NO_3^- reductase- which are encoded by the narGHJI operon and the napABC operon, respectively (Philippot and Højberg, 1999). Because NO_3^- reductase also functions as a NO_3^- respirer or reducer in the process known as dissimilatory reduction of NO_3^- to ammonia (NH_4^+) [DRNA], *nar*G and *nap*A genes do not necessarily represent the denitrifying bacteria (Philippot, 2002; Chèneby et al., 2003; Wallenstein et al., 2006). The reduction of NO_2^- to NO distinguishes denitrifiers from other NO_3^--respiring bacteria. This reaction is catalyzed by two different types of NO_2^- reductases (Nir), either a cytochrome cd1 encoded by *nir*S or a Cu-containing enzyme encoded by *nir*K. These genes are the first and most widely used molecular markers in the studies of denitrifying communities (Braker et al., 1998; Henry et al., 2004). In the next step, the NO reductase encoded by the *nor*B gene is responsible for conversion of NO to greenhouse gas N_2O. The last step of denitrification, from N_2O to N_2, is catalyzed by N_2O reductase and is encoded by the *nos*Z gene.

The N_2O is mainly produced through denitrification under anaerobic conditions while autotrophic and heterotrophic nitrification can also contribute to the N_2O flux (e.g. Inubushi et al., 1996; Canfield et al., 2010). An increasing number of studies have reported the distinct and significant role of nitrifier denitrification in the N_2O production from soil (Wrage et al., 2001; Venterea, 2007; Kool et al., 2011). However, it still remains elusive to what extent changes in N_2O production are explained by changes in the abundance of denitrifiers/nitrifiers, along with soil environmental factors.

Fire is one of the key drivers of the diversity and function of terrestrial ecosystems (Orians and Milewski, 2007) and one of the most important environmental changes that have been extensively studied (Guinto et al., 1999; Chen and Xu, 2010; Williams et al., 2011; Burton et al., 2011). Prescribed fire is widely used as a forest management tool to reduce fuel loads (Guinto et al., 1999; Pyke et al., 2010; Ryan et al., 2010). In general, fires immediately lead to the loss of C and N as gases and particulates into the atmosphere from the ecosystem, and to transformation of phosphorus (P) from organic to inorganic forms (Carter and Foster, 2004; González-pérez et al., 2004; Galang et al., 2010). Fire can also cause an immediate increase in N availability ($NO_3^- - N$ and/or $NH_4^+ - N$) (Wan et al., 2001; Carter and Foster, 2004). For example, Deluca et al. (2002) reported that N mineralization and nitrification rates decreased with time after last fire due to the increasing N immobilization with successional C loading in a fire chronosequence study. Fires can lead to a drastic reduction in soil microbial biomass in the short term and cause a shift in bacterial and fungal communities in forest soils (Prieto-Fernández et al., 1998; Pietikäinen et al., 2000; Bastias et al., 2006). Nevertheless, the understanding of the impact of long-term repeated prescribed burning on the interactive links of denitrifying communities, soil environmental factors and N_2O flux are still incomplete.

The objective of this study was to examine the effect of long-term repeated prescribed burning on soil N availability, *in situ* N_2O flux, and denitrification gene abundance in a wet sclerophyll forest. It was hypothesized: a) that long-term repeated prescribed burning would reduce soil N_2O flux by decreasing the abundance of denitrification genes and the N substrate availability; and b) a combination of soil N substrate availability, soil environmental factors (e.g. pH, moisture etc) and abundance of denitrification genes govern the N_2O flux from soils.

2. Material and methods

2.1. Experimental site

The research site is located in Peachester State Forest, southeast Queensland (26°50′S, 152°53′E) and was described in detail by Guinto et al. (1999). In brief, it is a native wet sclerophyll forest dominated by blackbutt (*Eucalyptus pilularis* Smith). Other canopy tree species include red bloodwood (*Corymbia intermedia* R. Baker), tallowwood (E. microcorys F. Muell.), red mahogany (E. resinifera Smith), turpentine (*Syncarpia glomufera* (Smith) Niedenzu) and brush box (*Lophostemon confertus* (R. Br.) P.G. Wilson & Waterhouse). The understory vegetation is variable and species-rich, in places dominated by grasses (e.g. *Imperata cyclindrica* (L.) Rauschel, *Digitaria ciliaris* (Retz.) Koeler), ferns (*Pteridium esculentum (*G. Forst.*) Cockayne, Blechnum cartilagineum* Sw), or shrubs (e.g. *Dodonaea triquetra* Andr., *Hibiscus heterophyllus* Vent., *Hovea acutifolia* Cunn. ex G. Don) (Lewis et al., in press). Average annual rainfall in this area is 1711 mm. Topography is undulating-to-rolling (2–16% slopes). The soil is deep and sandy having no perceptible increase in clay content to a depth of 60 cm. The soil is classified as yellow to red Kandosols (Isbell, 1996) (Alfisols, USDA classification).

The prescribed burning experiment was established in 1972 and consists of three treatments: (1)2 yearly burning (on average), (2) 4 yearly burning (on average) and (3) no burning. Prescribed fires were carried out in winter and are generally of low intensity (<2500 kW m^{-1}). There have been no wildfires at the site since 1969, no logging since the 1950s, and no fertilizer application or other silvicultural practices have been applied since the establishment of the burning experiment. There were four replicates for each treatment, with a total of 12 plots (30 × 27 m) randomly arranged across the experimental site. The latest burning for each treatment before sampling was conducted in 2007 (for 4 yearly burning plots) and 2009 (for 2 yearly burning plots). Therefore, this study sought to examine the long-term impacts of repetitive fire treatment on soil chemical and biological properties.

The atmospheric temperature in the experimental site ranged from 17.5 °C to 29 °C in January and 15.5 °C–26.5 °C in April, while the 7 day cumulative rainfall prior to the sampling was 39.3 mm (January) and 48.9 mm (April).

2.2. Soil sampling and analyses

Soil samples were taken in January and April 2011. For each sampling, approximately 10 cores of surface soils (0 – 10 cm) were collected using a corer (7 cm in diameter) from each treatment plot and combined to produce one composite sample. Soils were sieved to 4 mm immediately after sampling and stored at field moisture content in plastic bags at 4 °C prior to analysis (denitrification capacity was determined within 3 days). Subsamples for molecular analyses were sieved to 2 mm and stored at −80 °C.

Soil pH and electrical conductance (EC) were measured with a pH/EC meter using a soil-to-water ratio of 1:5. Soil moisture was determined gravimetrically by drying the soil at 105 °C for 24 h, and all results were expressed on an oven-dry basis. Microbial biomass C (MBC) and N (MBN) was determined by the chloroform fumigation extraction method as described by Vance et al. (1987) and