



Stimulation of long-term ammonium nitrogen deposition on methanogenesis by Methanocellaceae in a coastal wetland

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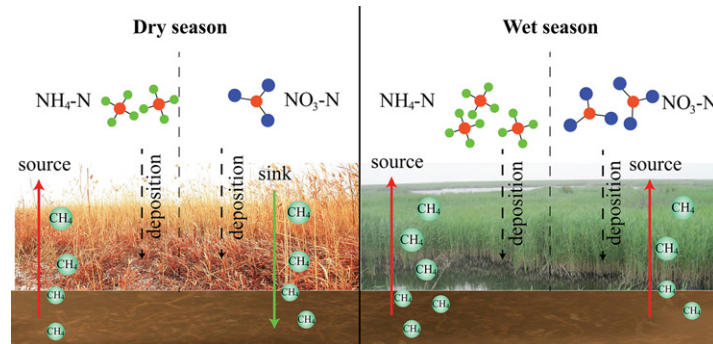
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HIGHLIGHTS

- The deposition of both ammonium and nitrate nitrogen on methane emission was tested.
- Deposition of ammonium nitrogen accelerated methane emissions all year round.
- Ammonium nitrogen converted Yellow River Delta to a CH₄ source during the dry season.
- Methanocellaceae increased in abundance in response to ammonium nitrogen deposition.
- Nitrate nitrogen deposition did not affect methane flux significantly.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 11 February 2017

Received in revised form 30 March 2017

Accepted 30 March 2017

Available online 5 April 2017

Editor: Elena Paoletti

Keywords:

Atmospheric nitrogen deposition
Ammonium and nitrate nitrogen
Methane flux
Methanogens
Dry-wet alternate
Coastal wetland

ABSTRACT

Atmospheric nitrogen deposition caused by human activities has been receiving much attention. Here, after long-term simulated ammonium and nitrate nitrogen deposition (NH₄Cl, KNO₃, and NH₄NO₃) in the Yellow River Delta (YRD), a sensitive coastal wetland ecosystem typified by a distinct wet and dry season, methane fluxes were measured, by adopting a closed static chamber technique. The results showed that deposition of ammonium nitrogen accelerated methane emissions all year round. Ammonium nitrogen deposition transformed the YRD from a methane sink into a source during the dry season. Methanocellaceae is the only methanogen with increased abundance after the application of NH₄Cl and NH₄NO₃, which promoted methane emissions, during the wet season. The findings suggested that Methanocellaceae may facilitate methane emissions in response to increased ammonium nitrogen deposition. Other methanogens might have profited from ammonium supplementation, such as Methanosarcinaceae. Deposition of nitrate nitrogen did not affect methane flux significantly. To the best of our knowledge, this study is the first to show that Methanocellaceae may be responsible for methane production in coastal wetland system. This study highlights the significant effect of ammonium nitrogen and slight effect of nitrate nitrogen on methane emission in the YRD and it will be helpful to understand the microbial mechanism responding to increased nitrogen deposition in the sensitive coastal wetland ecosystem.

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1. Introduction

Atmospheric nitrogen deposition caused by human activities has been receiving much attention (Kanakidou et al., 2016). The annual anthropogenic input of nitrogen in ecosystems has increased tenfold over the past 150 years, and it is predicted to be 2–3 times the current level in the 2050s (Sutton and Bleeker, 2013). Impacts by nitrogen deposition occur on a global scale, especially in terrestrial ecosystems (Asner et al., 2001; Matson et al., 2002). Meanwhile, nitrogen deposition affects various ecological types, such as marine (Zhang et al., 2010), forest (Zhang et al., 2016), grassland (Gomez-Casanovas et al., 2016), lake (Hobbs et al., 2016), and coastal (Pakeman et al., 2016) systems. Understanding of how nitrogen deposition affects coastal wetlands is limited, although this type of ecosystem is vulnerable to environmental change (Wolters et al., 2016).

The nitrogen cycle is closely coupled to the carbon cycle (Gruber and Galloway, 2008). The tight coupling between nitrogen enrichment and methanogenesis has evoked numerous studies assessing the application of nitrogen fertilizers and nitrogen deposition on methane emission (Bodelier, 2011). Liu and Greaver (2009) undertook meta-analyses of studies on wetlands as well as upland soils with treated and control sites which were comparable in terms of climatic, soil, and vegetation conditions. The conclusion was that N-enrichment of ecosystems in general would enhance methane emission because of lowering of consumption and increase of production. It is well known that effects of ammonium and nitrate nitrogen were different, and even the same type had distinct impacts on different regions (Bodelier, 2011). Therefore, it is of great significance to comprehensively study the effects of deposition of different types of nitrogen on the methane flux from vulnerable ecological regions.

All biogenic methane is produced by methanogenic archaea (Angel et al., 2012). Nitrogen deposition can change microbial community structure and function to influence methane production (Sinsabaugh et al., 2015), so understanding the response of methanogenic archaea may be the key to clarify the effect of different types of nitrogen enrichment on methane emission.

Atmospheric nitrogen deposition is one of the major nitrogen sources in the coastal zone of Yellow River Delta (YRD) (Ning et al., 2015), which has been shown to be a region of low adaptability and high environmental vulnerability (Wolters et al., 2016), typified by a distinct wet and dry season. Therefore, YRD is a natural system ideally suited to study the effect of increased nitrogen deposition on methane emissions. In this study, after long-term simulated atmospheric deposition of ammonium and nitrate nitrogen (NH_4Cl , KNO_3 , and NH_4NO_3) in the YRD, methane fluxes were determined to clarify whether there are different effects of ammonium and nitrate nitrogen on methane emission and the potential microbial mechanism responding to increased nitrogen deposition in the sensitive coastal wetland ecosystem.

2. Materials and methods

2.1. Sites, vegetation and environmental conditions

The study site ($37^{\circ}45'46''\text{N}$, $118^{\circ}58'40''\text{E}$) in the YRD (Fig. 1) has a temperate semi-humid continental monsoon climate (Yu et al., 2011). The average annual precipitation is 530–630 mm, concentrated mostly in summer. It is typified by distinct wet (in general, July to September) and dry (in general, October to June) seasons and remains inundated throughout the wet season. This research site is dominated by *Phragmites australis* interspersed with *Suaeda heteroptera* Kitag. Fluvo-aquic soil and saline soil are the main soil types and the soil texture is sandy loam.

2.2. Simulated nitrogen deposition

The description of simulated nitrogen deposition can be found elsewhere (Zhu et al., 2013). Briefly, the experiments (established in 2012)

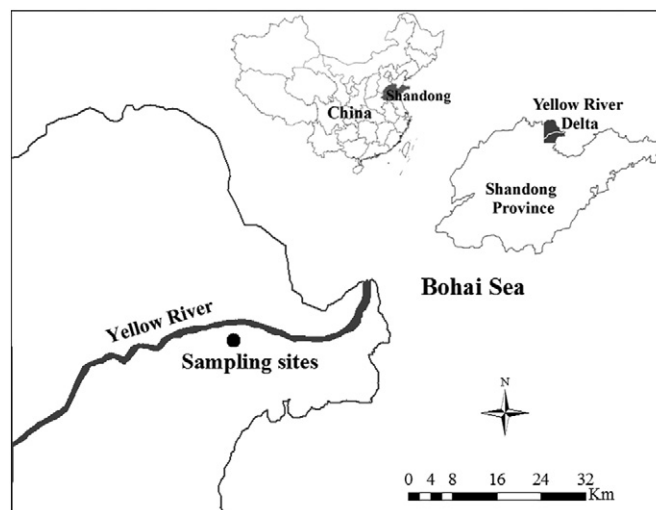


Fig. 1. Location of sampling sites in the Yellow River Delta.

were designed with three treatments, NH_4Cl , KNO_3 , and NH_4NO_3 . Each treatment had four replicated plots. For ammonium and nitrate nitrogen (NH_4Cl and KNO_3), each plot ($8\text{ m} \times 6\text{ m}$) receives nitrogen at a rate of $50\text{ kg N ha}^{-1}\text{ yr}^{-1}$. For NH_4NO_3 treatment, $100\text{ kg N ha}^{-1}\text{ yr}^{-1}$ is applied. These chemical reagents are dissolved in water and evenly sprayed over the area of interest: this process was carried out since 2012. The control treatment area is sprayed using pure water without any extra nitrogen source. These operations are performed once a month.

2.3. Methane flux determination

Boardwalks were installed in each plot to reduce soil disturbance during measurement. Methane fluxes were manually measured using the static chamber method as described by Wang and Wang (2003) and Zheng et al. (2008). A transparent Plexiglas® chamber (120 cm high) was placed on a stainless steel base ($50\text{ cm} \times 50\text{ cm}$) which was installed before the start of the experiment. A small fan was installed inside the chamber to mix the headspace gas and a rubber septum inserted into the chamber was used to collect gas samples. Five gas samples from the chamber headspace were collected at 15 min intervals with a syringe after enclosure of the chamber. The collected gas sample (50 ml) was immediately transferred into a 12-ml vacuum borosilicate vials (Labco, UK). The gas samples were analyzed with a gas chromatograph (GC) (Agilent 7890A, USA) equipped with a flame ionization detector and an automated flow-injection apparatus. Methane flux rates were calculated from the linear increase of methane mixing ratio in chambers, according to the description by Wang (2001). Briefly, that equation can be expressed as $F = \rho \frac{V}{A} \frac{P}{P_0} \frac{T_0}{T} \frac{dC_t}{dt}$, where F is the CH_4 flux rate, ρ is the CH_4 density at standard temperature and pressure, V is the volume of the chamber, A is the bottom surface area of the chamber, P and T are the air pressure and temperature in the chamber respectively, T_0 is 273.15 K and P_0 is 101.325 kPa , $\frac{dC_t}{dt}$ is average change rate of methane concentration. For the description of methane flux, if the region is a methane sink (methane flows from the atmosphere to below ground), the value is negative ($-$). If the region is a source of methane (methane flows from below ground to the atmosphere), the value is positive ($+$). If needed, $\sim 1.5\%$ (v/v of the headspace) difluoromethane (CH_2F_2) was added into the chambers to specifically inhibit methane oxidation before collecting the gas (Kruger et al., 2001). Then the gas was sampled and analyzed as above.

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