European Journal of Soil Biology 82 (2017) 81–87

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Effects of marsh cultivation and restoration on soil microbial communities in the Sanjiang Plain, Northeastern China

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ARTICLE INFO

Article history:
Received 8 March 2017
Received in revised form
9 August 2017
Accepted 23 August 2017
Available online 4 September 2017

Handling editor: Bryan Griffiths

Keywords:
Soil biogeochemical process
Agricultural abandonment
Wetland restoration
Wetland cultivation
Phospholipid fatty acid (PLFA)

ABSTRACT

To understand the impact of wetland cultivation and restoration on soil microbes and the corresponding soil biogeochemical processes, an experiment was established that included three cultivated treatments (marshes with 5, 15, and 25 years (CU05, CU15 and CU25) of soybean cultivation), two restored treatments (with 6 and 12 years (RE06 and RE12) of agricultural abandonment), and a natural marsh (NAT) as a reference. Changes in soil properties and microbial communities across the different treatments were analyzed. The results showed that soil microbial biomass and almost all the measured nutrients decreased in the order of NAT, restored treatments and cultivated treatments. Soil microbial structures in restored treatments were more similar to those of NAT than those of cultivated treatments. Specifically, the soil microbial communities of NAT and restored treatments were distinguished by a higher relative abundance of fungi, which were positively influenced by soil organic carbon and total nitrogen. In contrast, cultivated treatments were characterized by a higher relative abundance of gram-positive bacteria, which were positively influenced by the C/N ratio. The results indicated that the degraded soil in cultivated treatments began to recover after agricultural abandonment. However, the differences in soil microbial structures between restored treatment and NAT soils indicated that 12 years of restoration was not sufficient to restore the cultivated marshes to their natural status. Among all the measured soil properties, soil organic carbon and available potassium were identified as the main drivers of soil microbial communities, while the effect of pH on soil microbes was insignificant in this study. As available phosphorus was significantly decreased in the soil of restored treatments, phosphorus addition might be an effective way to accelerate the restoration process.

1. Introduction

Wetlands are important ecosystems that play a crucial role in cycling and sequestering carbon (C), nitrogen (N), and phosphorus (P) [1]. However, wetlands have changed dramatically in the past century. On the one hand, about half of global wetlands have been lost due to human disturbances [2]. On the other hand, due to a growing awareness of the important ecological and environmental functions of wetlands, the protection and restoration of wetlands have been implemented worldwide. These extreme changes in wetlands significantly impact the cycling, retention, and release of soil nutrients and further influence global biogeochemical cycles [3,4]. Thus, it is important to understand the effects of wetland changes on soil biogeochemical processes.

Soil microbes play a critical role in soil biogeochemical processes. Numerous studies have confirmed that land-use changes can profoundly affect soil microbial communities [5]. Thus, soil microbial communities can serve as a sensitive indicator of soil biogeochemical changes that are related to land-use changes [6]. A clear understanding of changes in microbial community structures would enhance our understanding of soil biogeochemical processes and greatly benefit land management and restoration [5,7]. However, changes in the soil microbes of marshes, caused by cultivation

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http://dx.doi.org/10.1016/j.ejsobi.2017.08.010
1164-5563/

and agricultural abandonment, and their link to soil biogeochemical processes have rarely been studied.

With regard to soil biogeochemical processes after wetlands change, many studies argue that it is difficult to reverse the degradation of wetlands. Although the plants and the hydrologic conditions could be restored to their natural states in a few years [8], the soil biogeochemical processes may still differ from those in natural wetlands, even after many years of restoration [3,9]. Some studies included soil microbial communities as sensitive indicators of how wetlands have changed relative to their natural state, and many similarities had been found between restoration wetlands and natural wetlands, which indicated that restoration wetlands had begun to recover [10,11]. However, few studies have reported that microbial communities have been fully re-established to their natural (or similar to natural) conditions, even after many years of restoration [10,12]. Peralta et al. (2014) suggested that microbial functions can be restored only when wetland restoration overcomes biological, physical, and chemical legacies that may restrain the restoration of soil microbes [13]. Thus, in order to provide a basis for wetland restoration and better understand the variation in soil biogeochemical processes after wetland changes, it is necessary to focus closely on the changes in soil microbial communities, as well as their relation to soil physicochemical properties.

Studies of soil biogeochemical processes in wetlands and other ecosystems show that land-use changes generate changes in soil physicochemical properties and also affect soil microbial communities [14]. Effects of land-use changes on soil physicochemical properties have been well studied; we know that land-use changes can have significant and long-lasting effects on soil carbon and nutrient content [15], soil texture [16], and pH [17,18] and that the effects largely arise from changes in the composition of plant species and associated management practices across land-use types [19]. However, less is known about how land use may affect the composition of microbial communities because we have a poor understanding of the relation between soil microbial compositions and soil physicochemical properties. The poor understanding mainly arises from the complex interactions between soil microbial communities and multiple soil physicochemical factors that are influenced by land-use changes [20]. Specifically, many soil factors, such as soil organic matter, soil type, salinity and pH [5,21–23], have a significant impact on soil microbial communities, and all of these soil factors may be altered by changes in land-use type.

To better understand these complex interactions, it is important to determine the main drivers of soil microbial communities. However, the main drivers of microbial community structures differ under different conditions and are unpredictable. Moreover, the mechanisms that underlie the changing main drivers have rarely been studied, impeding our understanding of soil biogeochemical processes.

To gain deeper insight into how soil biogeochemical processes are affected by wetland changes, we established a time-dependent experiment with cultivated treatments (marshes with 5, 15, and 25 years of soybean planting) and restored treatments (with 6 and 12 years of agricultural abandonment) in the Sanjiang Plain, which is a typical example of an inland freshwater marsh that is dramatically changing [24]. We examined changes in soil microbial communities and soil physicochemical properties in different wetlands. Our specific objectives were (i) to elucidate the changes in soil microbial communities in marshes that have experienced different periods of cultivation and agricultural abandonment, (ii) to investigate the relationship between microbial communities and soil properties, and (iii) to gain insight into soil biogeochemical processes after marsh changes and to provide a scientific basis for wetland restoration in the Sanjiang Plain.

2. Materials and methods

2.1. Site description

The study site is located at the Sanjiang Experimental Station of Wetland Ecology, Chinese Academy of Sciences (47°35′N, 133°31′E) in the Sanjiang Plain, China. *Calamagrostis angustifolia*-dominated marshes are one of the main wetland types in the Sanjiang Plain and occupy approximately 31% of the total wetland area [25]. This study site has a temperate continental monsoon climate. The mean annual precipitation is 566 mm (1990–2010), with approximately 50% falling in July and August. The mean annual air temperature is 2.5 °C, with a monthly mean temperature ranging from −20.4 °C in January to 21.6 °C in July.

2.2. Experiments and sample collection

Experiments were conducted on a freshwater marsh that had been previously dominated by *Calamagrostis angustifolia*. Six treatments, including three cultivation treatments, two restoration treatments and one reference, were established in a 2-km radius area. For each treatment, three replicates (40×40 m^2 plots) were randomly established. Three cultivation treatments (CU05, CU15 and CU25) have been implemented for soybean (*Glycine* max (L.) Merr.) planting since 1989 (CU25), 1999 (CU15), and 2009 (CU05), respectively. The two restoration treatments (RE06 and RE12) were established on soybean fields that had been agriculturally abandoned since 2003 (RE12) and 2008 (RE06), respectively. Undisturbed natural marsh (NAT) was identified as a reference. The experiments allowed us to follow changes in marsh soil over 5, 15, and 25 years of soybean cultivation and 6 and 12 years of restoration.

Soil samples were collected in June 2014 from 5 random points within each plot. At each point, the surface soil (0–20 cm) was collected from a 30×30 cm^2 area, and the five samples were composited. A total of 18 samples were collected, with 3 composite samples for each treatment. All soil samples that were free of major debris were divided into two parts. One part was designated for phospholipid fatty acid (PLFA) analysis and was immediately frozen in dry ice before being lyophilized in the lab. Another part was designated for nutrient analysis and was air-dried, sieved (<2 mm) and stored at room temperature. All sample analyses were conducted within 2 weeks of soil collection.

2.3. Nutrient analyses

Levels of SOC and TN were determined by dry combustion using a C/N analyzer (LECO Corporation, MI, USA). Soil pH was measured in a water-diluted soil solution (1:2.5). Samples equilibrated for 30 min before the pH measurements were recorded. Available nitrogen (AN), available phosphorus (AP) and available potassium (AK) were determined using the alkaline hydrolysis diffusion method, Bray-1 method and ammonium acetate extraction methods, respectively [25].

2.4. Phospholipid fatty acid analyses

PLFAs were extracted from lyophilized samples by the Bligh and Dyer (1959) [26] method with the modifications described by Bossio et al.(1998) [27]. Briefly, samples were extracted using a single-phase chloroform-methanol-citrate (1:2:0.8) buffer extractant. Phospholipids were separated from neutral lipids, and glycolipids on silicic solid-phase extraction columns (Supelco, Inc., Bellefonte, USA). After the polar lipids were methylated, PLFA methyl esters were analyzed using an Agilent 6890A Gas