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Soil microbial community responses to long-term land use intensification in subtropical grazing lands

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ABSTRACT

Land use intensification often results in modification in plant cover and nutrient inputs with subsequent potential effects on composition and structure of soil microbial community and fractions. The objective of this study was to understand the long-term (>22 yr) impacts of land use intensification (introduction of productive vegetation type, greater N fertilizer input and stocking rate) on soil microbial community composition and activity in Florida grazing lands. Experimental sites consisted of a gradient of management intensities ranging from native rangeland (lowest), silvopasture (intermediate), to sown pasture (highest). Increasing management intensity from native rangeland to sown promoted microbial biomass and activity. At the 0–10 cm, soil microbial biomass carbon (MBC) concentration was greater in sown pasture (334 mg kg^{-1}) compared to silvopasture and native rangeland (193 and 232 mg kg^{-1}, respectively). Similarly, potentially mineralizable C (PMC) increased in response to grazing land intensification (1.2 mg CO_2-C kg^{-1} d^{-1} for sown pastures vs. 0.5 and 0.6 mg CO_2-C kg^{-1} d^{-1} for native rangeland and silvopasture, respectively). Sown pastures exhibited the greatest levels of β-glucosidase activity (203 nmol g^{-1} soil $h.^{-1}$) and phospholipid fatty acid (PLFA) biomass (222 μmol kg^{-1} soil) compared to native rangeland and silvopasture. Results also demonstrated greater relative abundance of bacteria and less fungi as land use intensification increased from native rangeland to silvopasture or sown pasture. Our study indicated that long-term land use intensification affected the size, activity, and composition of soil microbial community in subtropical grazing lands.

1. Introduction

Soil microbial community mediates important biogeochemical processes controlling the global C cycle fluxes and stabilization of soil organic C (SOC) (Ingram et al., 2008). As soil microorganisms utilize SOC as a source of energy to sustain growth and reproduction, they also release significant amounts of CO_2 to the atmosphere. On a global scale, it is estimated that ~77 Pg C yr^{-1} is emitted to the atmosphere as result of autotrophic and heterotrophic soil respiration (Raich and Potter, 1995). The emission of CO_2 from terrestrial ecosystems is recognized as one of the largest fluxes in the global cycle and relatively small changes in microbial community and soil CO_2 patterns could lead to large impacts on the concentration of CO_2 in the atmosphere (Schlesinger and Andrews, 2000).

There is a general consensus in the scientific literature that soil microbial community is sensitive to changes in land use management. A number of studies have evaluated the impacts of land use management on soil microbial community responses in a variety of ecosystem types and climatic conditions (Steenwerth et al., 2002, 2006; McKinley et al., 2005; Bradley et al., 2006; Wang et al., 2006; Rousk and Bååth, 2007; Stark et al., 2008; Denef et al., 2009; Jangid et al., 2011; Vallejo et al., 2012; Yang et al., 2013). In addition to the impacts on microbial composition and structure, changes in vegetation cover or agricultural practices can also affect the amounts and characteristics of C stored in soils (McKinley et al., 2005; Perkins and Nowak, 2013). For instance, research has demonstrated that management practices intended to increase plant or animal production such as the use of nitrogen (N) fertilizer (Dawson et al., 2003; Denef et al., 2009) and improved grazing management (Abril and Bucher, 1999; Wang et al., 2006; Ingram et al., 2008; Liu et al., 2012; George et al., 2013; Raiesi and Riahi, 2014; Wang et al., 2014) can have major effects on soil microbial community abundance, composition, and activity with subsequent impact on soil C stocks.

The responses of soil microbial community to land use managements are often associated with changes in the quality and quantity of C substrates (Batten et al., 2006), particularly in the rhizosphere

Abbreviations: SOC, soil organic carbon; MBC, microbial biomass carbon; MBN, microbial biomass N; PMC, potentially mineralizable carbon; PMN, potentially mineralizable nitrogen; PLFA, phospholipid fatty acid.

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(Kowalchuk et al., 2002). For example, changes in plant species composition can affect labile C inputs such as root exudates, dead root cells, and fine root turnover (Shamoot et al., 1968). Similarly, previous studies have showed overgrazing can deplete high quality C substrate and, consequently, reduce microbial biomass and activity (Ingram et al., 2008). Root exudates may also respond to grazing intensity, which in turn, can affect the functional diversity and activity of soil microbial communities (Bardgett et al., 1998; Hamilton and Frank, 2001).

A number of techniques have been used to document the changes in soil microbial community responses to land use management. Microbial biomass C (MBC) and CO_2 efflux have been extensively used to characterize soil microbial responses. However, because of limitation associated with these techniques (e.g., MBC is not necessarily positively related to soil respiration) (Wang et al., 2003), additional procedures have been recently developed to evaluate responses of soil microorganisms to land use change. Phospholipid fatty acid analysis (PLFA), for instance, has been recognized as a rapid, and reproducible procedure that can be used to evaluate the impact of land use management on soil microbial community composition and diversity (Frostegård and Bååth, 1996). Although PLFA has been extensively used in different ecosystems and environments (Yao et al., 2000; Harris, 2003; Steenwerth et al., 2006; Denef et al., 2009; Inglett et al., 2011; Ford et al., 2013), one of its main limitation is the poor ability to determine some specific microbial groups(Frostegård et al., 2011). Similarly, enzyme activity has also been suggested as a sensitive indicator of microbial responses to different management practices (Dick, 1994; Bandick and Dick, 1999; Acosta-Martinez et al., 2008; Stott et al., 2010; Paudel et al., 2012; Olivera et al., 2014; Cui and Holden, 2015). Because soil microorganisms produce extracellular enzymes to catalyze the degradation of organic compounds, such as β-glucosidase for cellulose (Breznak and Brune, 1994), enzymes can be a sensitive indicator of microbial activity and their potential impacts on soil C dynamics (Turner et al., 2002).

An important aspect to be considered when evaluating microbial responses to land use management is the duration and intensity of soil disturbance or land use change. While soil microbial community structure may not be sensitive to short-term changes (Bradley et al., 2006), long-term studies provide important information regarding the direction and extent that soil microbes respond to shifts in land use management. Thus, the objective of this study was to investigate the long-term (>22 yr) impacts of grazing land intensification on soil microbial community responses.

2. Methods

2.1. Study area

The study was conducted at the University of Florida Range Cattle Research and Education Center in Ona, Florida (27°23′76″N, 82°56′11″W). The experimental site was characterized by subtropical climate with average annual precipitation of ~1206 mm, and average temperature of 21.5 °C. The predominant soil series included Ona fine sand (sandy, siliceous, hyperthermic Typic Alaquods,) and Smyrna sand (sandy, siliceous, hyperthermic Aeric Alaquods). The study was conducted on grazing land ecosystems that represent a gradient of management intensities ranging from native rangeland (lowest), silvopasture (intermediate), to improved bahiagrass pasture (highest). Each field replicated site (~6 ha; n = 2) was adjacent to each other but managed independently. All sites exhibited the same topography, soil series, and climate conditions, and have been established and consistently maintained for over 20 years. In the context of this study, increased management intensity was defined as the introduction of more productive non-native plant species, greater N input, and greater stocking rate. More detailed information about the experimental sites is presented in Silveira et al. (2014). The predominant vegetation in the rangeland consists of shrubs and perennial grasses, including saw palmetto (*Serenoa repens* Bartr.), chalky bluestem (*Andropogon capillipes* Nash), broomsedge bluestem (*Andropogon virginicus* L.), and creeping bluestem (*Schizachyrium stoloniferum* Nash. Silvopasture consisted of a 12-yr-old stand of slash pine (*Pinus elliottii* Engelm) trees planted into a Pensacola bahiagrass (*Paspalum notatum* Flueggé) pasture while sown pastures were 31-yr-old stand of bahiagrass.

2.2. Soil sampling, microbial biomass and potentially mineralizable C and N determination

In each experimental unit, five (20 × 20 m) quadrats were established (~75 m apart) along a diagonal transect. Eight random soil cores (2.2 cm diameter) were collected (0 to 10 and 10 to 20 cm depths) from each sampling quadrat and composited within soil depth. Soil sampling occurred in the summer (September 2012) and was repeated in the winter (January/February 2013). Immediately after collection, soil samples were placed in plastic bags and stored in a cooler with ice until transported to the lab. Coarse roots and rocks were removed and soil moisture concentration was determined by oven-drying a sub-sample at 105 °C for 48 h. Samples were divided into two subsamples and stored separately at either 4 °C or −20 °C.

Microbial biomass C and N (MBN) concentrations were estimated using the chloroform fumigation-extraction method (Vance et al., 1987). Briefly, one set of subsamples (2.5 g soil each) C was equilibrated with 25 mL of 0.5-M K_2SO_4, shaken for 1 h on a longitudinal shaker, and vacuum-filtered through #41 Whatman filter paper. The second set was fumigated with ethanol-free chloroform (24 h; 25 °C) before extraction. Total organic C (TOC) in the extracts was measured in a Shimadzu TOC-5000A analyzer (Columbia, Maryland). Microbial biomass C concentration was calculated by subtracting the extractable TOC in non-fumigated soils from extractable TOC in chloroform-treated soil. A set of subsamples of the extracts were digested with sulfuric acid and copper sulfate mixture (EPA method 351.2; United States Environmental Protection Agency, 1993) and analyzed for NH_4-N concentration using a Technicon AutoAnalyzer (Seal Analytical, Mequon, Wisconsin) for microbial biomass N determination. Microbial biomass N was calculated as described for MBC.

Potential C mineralization rate was estimated using the laboratory incubation method (Zibilske, 1994). Approximately 5 g of fresh, moist soil was placed into a 150-mL flask, and incubated in the dark at 25 °C for 10 days. Soil moisture concentration was maintained at 60% water-filled pore space to maximize aerobic microbial activity (herein determined by CO_2 production). The CO_2 concentration in the headspace was measured on days 1, 2, 3, 4, 7, 8, 9, and 10. At each sampling date, a 500-mL gas sample was collected from the headspace and injected into a PerkinElmer Clarus 400 gas chromatograph (Waltham, Massachusetts). The gas chromatograph was equipped with a Poropak N (Supelco, Bellefonte, Pennsylvania) column and thermal-conductivity detector (TCD) for CO_2 analysis. A linear regression between CO_2 concentration and incubation time was used to calculate the potential C mineralization rate.

Potentially mineralizable N (PMN) was estimated using the anaerobic incubation procedure (White and Reddy, 2000). Briefly, 2.5 g of soil received 5 mL of distilled de-ionized (DDI) water and was incubated under anaerobic conditions at 40 °C for 10 days. After the incubation, 20 mL of 2-M KCl solution was added to the samples and the slurry was shaken on a longitudinal shaker for 1 h. Suspension was transferred to centrifuge tubes and centrifuged for 10 min at 6000 rpm. The solution was then filtered through a Whatman #41 filter paper and analyzed for NH_4-N concentrations. Another set of subsamples (2.5 g soil) was reacted with 25 mL of 2-M KCl solution to obtain extractable NH_4 prior to incubation. All extracts were analyzed for NH_4-N on an AQ_2 discrete analyzer (SEAL Analytical, Inc., Mequon, Wisconsin). Potentially mineralizable N was calculated as the difference in NH_4-N concentration before and after incubation.