Soil Biology & Biochemistry 103 (2016) 446–451

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Plants modify the effects of earthworms on the soil microbial community and its activity in a subtropical ecosystem

Meirong Lv [a, c], Yuanhu Shao [b, c], Yongbiao Lin [c], Chenfei Liang [d], Jun Dai [e], Yan Liu [a], Pingping Fan [a], Weixin Zhang [b, c, *], Shenglei Fu [b, c, **]

[a] Institute of Oceanographic Instrumentation, Shandong Academy of Sciences, Qingdao, 260000, China
[b] College of Environment and Planning, Henan University, Kaifeng, 475001, China
[c] Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, 510650, China
[d] Zhejiang Provincial Key Laboratory of Carbon Cycling in Forest Ecosystems and Carbon Sequestration, School of Environmental and Resource Sciences, Zhejiang A&F University, Lin'an, Hangzhou, 311300, China
[e] The College of Natural Resources and Environment, South China Agricultural University, Guangzhou, 510642, China

A R T I C L E I N F O

Article history:
Received 7 April 2016
Received in revised form
6 September 2016
Accepted 27 September 2016

Keywords:
Earthworm
Plant
Labile C
Available P
Enzyme activity
Soil respiration

A B S T R A C T

To illustrate the interplay between top-down and bottom-up forces on the soil microbial community and activity, we conducted a 3-year field experiment investigating the interactions of the dominant, exotic earthworm *Pontoscolex corethrurus* and the native plant *Evodia lepta* in subtropical soil. We found that both earthworms and plants were able to regulate soil carbon (C) and phosphorous (P) dynamics by affecting the soil fungi-to-bacteria ratio, as well as the labile C and available P content. The increase in soil respiration in plots with only earthworms was mainly due to the increased proportion of soil bacteria that had a fast turnover and low assimilation efficiency. The increase in soil respiration in plots with only plants was possibly due to an increase in the metabolic quotient of soil microorganisms under the stress of soil available P. The significant decrease of soil available P and increase in soil phosphatase activity indicated an intensified demand for soil available P when plants were present; accordingly, earthworms' effect on soil available P was only significant in plots with plants. Overall, when plants were present, the soil fungi-to-bacteria ratio (F/B) and C dynamics changed in a similar way regardless of the reduction of the earthworm populations. The earthworms' effects on soil P processes were enhanced by the presence of plants. These indicated that plants were one of the key regulators of the effects of earthworms on the soil microbial community and its activity in this subtropical ecosystem.

1. Introduction

Soil microorganisms play key roles in the biogeochemical transformation of soil organic matter and nutrients (Massenssini et al., 2015). Soil fungi decompose organic material and retain soil nutrients from leakage by facilitating macroaggregate formation and stabilization (Ansari et al., 2013). In addition, some soil fungi establish mutualistic associations with roots, improving plant nutrient absorption and growth (Yaseen et al., 2011). As fast-growing r-strategists, bacteria utilize readily available C and accelerate the transformation of CO_2 due to their high turnover rate (5.9 days, Bååth, 1994) and low C assimilation efficiency (5–10%, Sakamoto and Oba, 1994). As the basal species of the soil food web, soil microorganisms have been argued to be controlled by bottom-up or top-down forces (Wardle and Yeates, 1993). Earthworms can affect soil microorganisms through the stimulating effect of their mucus (Chapuis-Lardy et al., 2010), as well as through their feeding activity. When soil microorganisms pass through the earthworm gut, some species proliferate, while others can not survive due to the gut conditions (e.g., abundant soluble organic C, microbicidal substances and more anaerobic conditions), leading to a decrease in the fungi-to-bacteria ratio (Brown, 1995; Lavelle et al., 1995). That is, through their feeding activity, earthworms could exhibit top-down control of soil microorganisms.

* Corresponding author. College of Environment and Planning, Henan University, Kaifeng, 475001, China.
** Corresponding author. College of Environment and Planning, Henan University, Kaifeng, 475001, China.
E-mail addresses: weixinzhang@139.com (W. Zhang), fsl@henu.edu.cn (S. Fu).

http://dx.doi.org/10.1016/j.soilbio.2016.09.020

Although the fungi-to-bacteria ratio decreased when soil microorganisms passed through earthworm guts, no significant change in the ratio has been found in earthworm-treated soils in previous studies (Pawlett et al., 2009). How might this paradoxical phenomenon arise? It has been reported that top-down forces controlled the biotic community and exhibited significant ecological effects when bottom-up forces were weak; likewise, bottom-up forces could have controlled the biotic community when top-down forces were weak (Frank et al., 2007). These studies indicated that bottom-up forces could modify soil animal effects (top-down effects) on the soil microbial community. Plants can change soil nutrient availability by supplying high-quality carbon from their roots and litter or by competing for nutrients, exhibiting bottom-up forces (Bardgett et al., 2005). As an integral part of the ecosystem, plants have always been used in experiments focusing on the ecological effects of earthworms (Groffman et al., 2004; Fonte and Six, 2010); however, the effects of the interplay between plants and earthworms on the soil microbial community are not understood.

In the present study, we conducted a 3-year field experiment to investigate the effects of the interactions of the native plant *Evodia lepta* and the dominant exotic earthworm *Pontoscolex corethrurus* on the microbial community of a subtropical soil in China. We hypothesized that 1) earthworms would change the soil microbial community and its activity according to their feeding activity as a top-down force; 2) plants would change the soil microbial community and activity by changing soil carbon and nutrient availability as a bottom-up force; and 3) plants would modify the effects of earthworms on the soil microbial community and activity.

2. Methods

2.1. Experimental design

A long-term experiment was performed at the Heshan Field Research Station of the Chinese Academy of Sciences (22°41′N, 112°54′E), Guangdong, China. The region has a typical subtropical monsoon climate. The mean annual precipitation is 1700 mm, and the average temperature is 21.7 °C. The soil is an Acrisol. An area of 35 m × 10 m was selected at our study site in an *Acacia auriculaeformis* plantation, where the canopy coverage was approximately 50%. The understory vegetation was dominated by *E. lepta*, *Rhodomyrtus tomentosa*, *Litsea cubeba*, *Ilex asprella*, and *Dicranopteris linearis*. The soil macrofauna was dominated by the endogeic earthworm *Pontoscolex corethrurus*.

Sixteen plots (1 × 2 m) were established in an 8 × 20 m area. The plots were isolated from each other and from the surrounding soil by inserting PVC boards into the soil along the plot borders to a depth of 80 cm, leaving a rim of 20 cm above the soil surface. At the beginning of the study, in all of the plots, the earthworm population was reduced by electrical shock (Staddon et al., 2003), and all plants were removed. The plots were then divided into four treatments: CK, no earthworms and no plants were reintroduced; EW, earthworms were reintroduced; PL, plants were reintroduced; and EWPL, both earthworms and plants were reintroduced. The treatments were arranged in a randomized design, with four replicates per treatment. In 2007, three seedlings of *E. lepta*, a shrub and herbaceous plant belonging to the family Rutaceae that is widely distributed in many areas of Southeast Asia (Sichaem et al., 2014), were transplanted into each plot in which plants were to be reintroduced. In May 2009, 200 *P. corethrurus* earthworms were inoculated into each plot in which earthworms were to be reintroduced. This geophagous earthworm is a peregrine pantropical species and is polyhumicendogeic, inhabiting the first 30 cm of soil and adapting to a wide range of soil pH, organic matter content and textures (Ayala and Barois, 2015). Earthworms were obtained from the surrounding soil and added to the plots at a soil depth of approximately 10 cm. The number of *P. corethrurus* added was consistent with the earthworm density found in the surrounding soil. We assumed that the earthworm population was successfully reduced by the electric shock since, after conducting them, we did not observe obvious fresh earthworm casts in the CK or PL plots.

2.2. Soil sampling and analysis

In April 2012, soil cores to a depth of 10 cm were obtained from six randomly selected points in each plot. The six cores were combined to make one composite soil sample per plot. The composite sample was passed through a 2-mm sieve and stored for the future determination of the soil microbial and biochemical properties. Phospholipid fatty acids (PLFAs) were analyzed using the method described by Bossio and Scow (1998). The PLFAs were quantified by comparing their peak areas with those of an internal standard, C19:0. All of the PLFAs were considered to represent the entire soil microbial community. Bacteria were represented by 12 PLFAs (i15:0, a15:0, 15:0, i16:0, 16:0, i17:0, a17:0, cy17:0, 17:0, 16:1ω7c, 18:1ω7c, cy19:0); and fungi were represented by 3 PLFAs (18:1ω9c, 18:2ω6,9c and 18:3ω6,9,12c). Microbial biomass carbon was determined using the chloroform fumigation extraction method (Vance et al., 1987). Soil respiration was assessed by measuring the CO_2 evolution from soil samples during a 24 h incubation at 25 °C (Anderson and Domsch, 1973). The respiratory quotient was calculated as the amount of CO_2-C produced per unit of microbial biomass C (Thirukkumaran and Parkinson, 2000). The activities of two extracellular enzymes, β-glucosidase for C cycling and acid phosphatase for P cycling, were determined according to established procedures (Iyyemperumal and Shi, 2008) with the substrates *p*-nitrophenyl-β-D-glucopyranoside and *p*-nitrophenyl-phosphate, respectively. Soil organic matter was determined by wet digestion according to the Turin method (Nelson and Sommer, 1975), dissolved organic carbon and nitrogen by the K_2SO_4-extracted method (Bes et al., 2010) and soil available P by the Bray method (Bray and Kurtz, 1945).

2.3. Statistics

To examine the effects of earthworms on the soil microbial and chemical properties with or without plants, one-way ANOVA was performed. The means were compared by the LSD test. Statistical analyses were performed using SPSS 16.0.

3. Results

3.1. Soil chemical properties

Treatment with earthworms alone did not significantly affect soil available P, whereas treatment with plants alone decreased soil available P significantly compared with the CK treatment. When plants and earthworms were both present, soil available P was not significantly different than that in the CK treatment (Table 1). No significant differences in soil organic matter (SOM), soil dissolved organic carbon (DOC) and soil dissolved nitrogen (DN) were found among all treatments.

3.2. Soil microbial properties

No significant differences were found among treatments for soil microbial biomass measured by either the chloroform fumigation extraction method or PLFA analysis (Fig. 1a and b). Earthworms alone significantly decreased the soil fungi-to-bacteria ratio (Fig. 1c). Earthworms alone also significantly increased soil