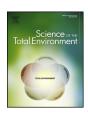
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Biochar increases plant growth and alters microbial communities via regulating the moisture and temperature of green roof substrates



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HIGHLIGHTS

- The effects of biochar application on soil and plants were studied in green roofs.
- Biochar application increased soil moisture and reduced temperature of green roof.
- Biochar application can largely prompt plant growth and alter microbial community.
- The application rate of 10–15% sludge biochar exerted the most significant effects.
- The ecological benefits of green roof can be greatly raised by biochar application.

GRAPHICAL ABSTRACT



The applications of sludge biochar will affect the plant growth and microbial community via regulating soil properties and further increasing ecological benefits of green roof.



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ABSTRACT

Green roofs have increasingly been designed and applied to relieve environmental problems, such as water loss, air pollution as well as heat island effect. Substrate and vegetation are important components of green roofs providing ecosystem services and benefiting the urban development. Biochar made from sewage sludge could be potentially used as the substrate amendment for green roofs, however, the effects of biochar on substrate quality and plant performance in green roofs are still unclear. We evaluated the effects of adding sludge biochar (0, 5, 10, 15 and 20%, v/v) to natural soil planted with three types of plant species (ryegrass, *Sedum lineare* and cucumber) on soil properties, plant growth and microbial communities in both green roof and ground ecosystems. Our results showed that sludge biochar addition significantly increased substrate moisture, adjusted substrate temperature, altered microbial community structure and increased plant growth. The application rate of 10–15% sludge biochar on the green roof exerted the most significant effects on both microbial and plant biomass by 63.9–89.6% and 54.0–54.2% respectively. Path analysis showed that biochar addition had a strong effect on microbial biomass via changing the soil air-filled porosity, soil moisture and temperature, and promoted plant growth through the positive effects on microbial biomass. These results suggest that the applications of biochar at an appropriate rate can significantly alter plant growth and microbial community structure, and increase the ecological benefits of green roofs via exerting effects on the moisture, temperature and nutrients of roof substrates.

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1. Introduction

Rapid urbanization has caused serious environmental problems such as water loss, air pollution, habitat fragmentation, biodiversity reduction,

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as well as urban heat island effect (Grimm et al., 2008; Walsh et al., 2005). To relieve these environmental problems, green roofs have increasingly been designed and applied (Oberndorfer et al., 2007). Green roofs are roofs that are partially or completely covered with vegetation and growing medium, providing important environmental and economic benefits for individuals, businesses, communities (Oberndorfer et al., 2007). The ecological benefits of green roofs include the creation and restoration of natural habitat, filtration of acid rain, heavy metals and air pollutants, reduction of energy consumption of building, and mitigation of storm water run-off and heat island effects (Getter et al., 2009; Lundholm, 2015; Mcguire et al., 2013; Onmura et al., 2001; Santamouris, 2014; VanWoert et al., 2005).

The ecological benefits of green roofs are dependent upon substrate quality because substrates support plant growth and provide habitat for microorganisms. However, the green roof environment has been facing several ecological challenges including drought, low nutrients, high temperatures and thin substrate layers (Eumorfopoulou and Aravantinos, 1998; Rayner et al., 2016). Therefore, increased nutrient availability, drainage, and substrate quality are important for green roof plant establishment and growth (Chan et al., 2008; Hossain et al., 2010; Kookana et al., 2011).

Biochar made from a wide range of raw materials, including straw, livestock manure and municipal solid waste (sewage sludge) has been broadly applied in horticulture and agriculture (Yue et al., 2017). Biochar can increase soil organic matter content, improve the drainage and permeability of soil, improve water and nutrient retention increase cation exchange capacity (CEC), and adjust micro-environmental climate (Glaser et al., 2002; Kolton et al., 2011; Lehmann et al., 2003). By exerting direct effects on soil quality, biochar could further regulate plant growth and microbial community structure (Fierer and Lennon, 2011; Lehmann et al., 2003; Laird et al., 2010; Lennon et al., 2012).

Sludge biochar has been proposed as a useful soil amendment because it is of effectiveness in improving soil properties such as soil structure, infiltration rates, water holding capacity, soil respiration, pH, fertility, and promoting plant growth (Hernández-Apaolaza et al., 2000; Méndez et al., 2012; Sort and Alcañiz, 1999; Silber et al., 2010; Song et al., 2014). Sludge biochar can also increase the availability of trace elements for plants and soil microorganisms, decrease the bioavailability of potentially toxic elements in soils and suppress the uptake and translocation of these contaminants by plants (Ishii and Kadoya, 1994; Khan et al., 2013; Steiner et al., 2008; Zhang et al., 2006). Moreover, sludge biochar may enhance soil carbon pools (Borchard et al., 2014; Wang et al., 2015) and reduce the emission of greenhouse gases such as CH₄ and N₂O (Joseph et al., 2010; Mukherjee et al., 2014).

Given the stressful environment of the green roof, sludge biochar may be an important amendment to green roof substrate, there is however a lack of information on the response of plants and soil microbial communities to biochar application in the green roof system. We conducted a comparative experiment to detect the ecological benefits of sludge biochar on green roofs by comparing substrate properties, microbial communities and plant growth, species diversity index and richness in different ecosystems (green roof and ground). Here, microbial communities were investigated because soil microbe is a vital ecosystem component determining nutrient recycling and supporting successful establishment of plants (Lavelle et al., 2016; Molineux et al., 2014). We asked the following specific questions:

- 1) What are the effects of biochar application on the properties of green roof substrate (temperature, moisture and nutrients)?
- 2) How does biochar application influence the performance of plants and microbial communities in green roofs?

2. Materials and methods

2.1. Study area and experimental design

This experiment was carried out on the roof (28 m above ground) and ground of the building of College of Recourse and Environment in

Nanjing Agriculture University, China (118°85′E, 32°04′N). This region has a subtropical monsoon climate, with an average annual precipitation of 1106.5 mm and a relative humidity of 76%. The mean temperature is 33.5 °C in summer (from June to August), and 1.2 °C in winter (from December to February).

Five treatments with five replicates were utilized in both the roof and ground experimental ecosystems including natural soil (CK), the mixture of local natural soil and 5% sludge biochar (5% SB), 10% sludge biochar (10% SB), 15% sludge biochar (15% SB), and 20% sludge biochar (20% SB) respectively (Fig. A.1). The natural soil was obtained from an alluvial soil at Pukou, Nanjing, Jiangsu, China. The sludge biochar was derived from sludge sewage (provided by sewage treatment factory in Jinhua, Zhejiang, China) with a pyrolysis temperature of 600 °C. The initial properties of natural soil (Yellow-brown soil), sludge biochar and the mixture of local natural soil with sludge biochar are summarized in Table 1. The five treatments on the green roof and ground are shown in Fig. 1, referred from Luo et al. (2015) and Cao et al. (2014). During the experiment, the five treatments were randomly replaced monthly to minimize the block effects.

Five vertical layouts were set up for the experiment, including the plant layer, substrate layer, filter layer, drainage layer and waterproof layer. The average thickness of substrates was 25 cm. Both green roof and ground were divided into five 1 m \times 1 m areas. Each area was separated into three districts (0.3 m \times 1 m) by waterproof layer (3 cm thick). Each of ten individuals of three plant species, including ryegrass (Lolium perenne L.), sedum lineare (Sedum lineare Thunb.) and cucumber (Cucumis sativus L.) were planted in each district (Fig. 1). The seeds of the three plants were purchased from Jiangsu Agriculture Science Academy, Nanjing, Jiangsu Province, China.

2.2. Sampling and pretreatment

The experiment was performed from June to December in 2015. Three districts were randomly sampled per 30 days. The soil temperature was detected at 15 cm depth. Temperature was continuously measured for 5 consecutive days (from July 25 to August 1 in summer, and from November 25 to 29 in winter) at 10:00 am. Both substrate moisture and plant biomass data were collected when the temperature was measured. The above-ground and below-ground plant biomass was harvested, cleaned and dried at 75 °C for 48 h for biomass measurement (dry weight).

2.3. Sample measurements and analyses

An analytical balance was used to measure substrate moisture and plant biomass (Denver, T214). Temperature and pH were measured using a portable pH meter (IQ 150 United States) and its temperature probe. The drying bulk density (DBD), saturated bulk density (SBD), water holding capacity (WHC) and air-filled porosity (AFP) were measured as described by Handreck and Black (2010). DBD and SBD were determined by dividing the weight of saturated substrate and dry substrate to the volume. AFP was determined as the difference of one minus and the ratio of volume-weight and initial weight. In the drought stress experiment, the substrate moisture and temperature data were measured at the surrounding temperature maximum and minimum. The appearance of plant wilting was monitored in the drought period. Wilted plants were put in a dark and wet box (relative humidity 80%) to verify whether the plant was permanently wilted or not. If plants recovered turgor, the treatment would be repeated until plants reached the permanent wilting point. Plant available water contained in substrate was determined during the drought stage. Plant available water was determined as the difference between the soil moisture at the permanent wilting point and field capacity. Phospholipid fatty acids (PLFAs) were extracted in triplicate from each soil sample using a modified Bligh-Dyer extraction method (Frostegård and Bååth, 1996). The fresh dry soil samples of 8 g $(-70 \, ^{\circ}\text{C})$ were extracted, and the

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