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Analysis of the occurrence and activity of diazotrophic communities in organic and conventional horticultural soils

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ABSTRACT

Nitrogen (N)-fixing microorganisms are an important soil component as they help replenish the pool of available N. Organic management can influence the N-fixing community; however, diazotroph community structure and activity in horticultural systems and the impacts of specific cultivation methods (*i.e.*, greenhouse and open field) are unclear. Using the *nifH* gene, a marker gene for the microbial community involved in N fixation, we investigated the occurrence (DNA) and activity (RNA) of the diazotrophic community in organically and conventionally managed soils in a horticultural system over the course of 1 year. Ordination analysis of DGGE profiles revealed organic management affected the community structure in the greenhouse but not the open field; fertilization intensity may explain this divergent response, as indicated by the relevance of total C content to community structure. Quantitative PCR revealed that organic management increased the abundance and activity of diazotrophs. The soluble organic N concentration was higher in organically managed soils and during warmer months, and correlated with diazotroph abundance. Most identified sequences were from known diazotrophs, predominantly β-, γ- and α-proteobacteria. Twenty-four bands resembled *Pseudomonas stutzeri* and eight resembled *Azoarcus* sp. Our results show that the cultivation method controls the extent of the effects of season and organic management on diazotrophs, and that greenhouse cultivation can boost the effects of organic management on this community. Organic management intensified the positive effect of seasonal temperature on diazotroph abundance and activity, which may increase biological nitrogen fixation rates. In tandem, soil DNA and RNA analyses provide a comprehensive picture of the community.

1. Introduction

Biological nitrogen fixation (BNF) is the reduction of atmospheric nitrogen to bioavailable ammonium by microorganisms. BNF is catalyzed by the enzyme nitrogenase and is performed by a diverse group of prokaryotic microorganisms that harbor the nitrogenase reductase (*nifH*) gene, which encodes the iron protein subunit of nitrogenase (Zehr et al., 2003). BNF is important in terrestrial N cycling, contributing 90–130 TgN per year (Tg = 10^{12} g) to the biosphere (Galloway et al., 1995), and is mainly performed by bacteria that live in association with leguminous plants. However, free-living diazotrophs in soils have received increased attention due to their important N contribution (Cleveland et al., 1999). In agroecosystems, the structure and ability of free-living diazotroph assemblages to fix N depends upon the management practice (DeLuca et al., 1996; Reed et al., 2007). Therefore, an increased understanding of the impacts of different management practices on diazotroph communities would provide knowledge to optimize the activity of these organisms.

As an alternative to conventional management, organic management recognizes the biological and economic importance of microbially mediated processes (Girvan et al., 2004). Organic systems rely on N mineralization from organic inputs, which can limit soil N availability for plant uptake and growth (Reeve et al., 2008); therefore, the microbial community that mediates N fixation may be critical for enhancing the sustainability of organic systems. The effects of organic management on the diazotrophic community have been previously studied in barley-bean systems (Orr et al., 2011) and rice paddy systems (Wang et al., 2012). In contrast to extensive cropping systems, horticultural systems further intensify the use of farmland in both space and time by increasing inputs (*i.e.*,

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fertilizers) and the use of greenhouses. Organic agriculture is a low input management practice, which has been recognized to reduce the adverse effects of conventional agriculture on the microbial community (Girvan et al., 2003; Verbruggen et al., 2010; Widmer et al., 2006); however, little is known about the effect of organic agriculture on the diazotroph communities in horticultural soils.

PCR-denaturing gradient gel electrophoresis (DGGE) banding pattern analysis is widely used for evaluation of microbial community structure, and sequencing of the bands of interest can help to determine the taxonomic structure of the microbial community (Muyzer and Smalla, 1998). Due to DGGE biases (*i.e.*, co-migration and dispersal of dominant amplicons in the gel), gel profile analyses can be complemented with sequencing and phylogenetic analyses to further assess changes in the population compositions observed in DGGE profiles (Wartiainen et al., 2008). This approach proved to be a robust method for determining the phylotype composition of a diverse diazotroph assemblage (Lovell et al., 2008). However, molecular studies targeting microbial DNA have a number of potential disadvantages, such as the co-extraction and amplification of DNA molecules that can remain intact in the soil matrix for long periods of time after bacterial cell death (Kowalchuk et al., 2006), and therefore analysis of DNA alone may not accurately describe the influence of the environment on microbial communities (Hoshino and Matsumoto, 2007). RNA is less stable in soil than DNA, and consequently, RNA-based measurements of the microbial response may be more sensitive for revealing short-term changes in the bacterial community (Hoshino and Matsumoto, 2007) as well as long-term environmental changes (*i.e.*, management type or land use) (Girvan et al., 2004). Additionally, the transcript levels of protein-encoding genes provide an acceptable approximation of the relative activity of the encoded function (Kowalchuk et al., 2006).

We investigated the diazotrophic community in an organic and conventional horticultural system under two different cultivation methods, *i.e.*, greenhouse and open field. Separating the effects of agricultural management from natural fluctuations (*i.e.*, due to temperature and rainfall) is critical when investigating soil microbial communities (Lauber et al., 2013); therefore, we also studied the seasonal variations in the diazotrophic community over four seasons in a single year. Soil DNA and RNA were analyzed in tandem to construct and quantify the community structure profiles.

2. Materials and methods

2.1. Experimental site and soil sampling

The study area is an experimental horticultural farm located in the suburbs of Shanghai (30°57′ N, 121°21′ E), China. The climate is humid subtropical, and 70% of the annual precipitation (1255 mm) occurs between May and September. The mean annual air temperature is 17.5 °C, and the average total annual sunshine is 1778 h. The soil is a fluvisol developing toward a cambisol and contains approximately 8.3% sand, 70.7% silt and 21% clay to a soil depth of 40 cm. The organically and conventionally managed fields are spatially separated within the same farm. The organically managed fields, under this type of management since 2003, are fertilized with composted chicken manure at an average rate of 12 ton ha^{-1} $crop^{-1}$. The conventional fields (under conventional management for more than 30 years) use an NPK compound fertilizer (14:16:15) at an average rate of 750 kg ha^{-1} $crop^{-1}$ and urea at a rate of 225 kg ha^{-1} $crop^{-1}$. Four to five crops per year are planted in the greenhouses and three per year in the open field. The same numbers of crops are cultivated in the organic and conventional fields per year. The main crops cultivated are spinach (*Spinacia oleracea*), pepper (*Capsicum annuum*), onion (*Allium cepa*), celery (*Apium graveolens* var. *dulce*), Chinese cabbage (*Brassica rapa pekinensis*), coriander (*Coriandrum sativum*), bean (*Glycine max* and *Vicia faba*), bok choi (*Brassica rapa chinensis*) and garlic (*Allium sativum*). Legumes are included in the rotation of both the organic and conventional fields. Weeds are controlled manually in the organic fields and chemically in the conventional fields (the most common herbicides used are glyphosate and linuron in dosages as recommended by the vendor). Cultural practices (*e.g.*, rotation and intercropping) are sufficient to control pests and diseases in the organic plots and synthetic pesticides are used in the conventional plots (the most common pesticides used are tebuconazole, mandipropamid, chlorpyrifos and spinosad in dosages as recommended by the vendor). Furrow irrigation is employed with the aim of maintaining adequate soil moisture for the needs of the crop; the volume and frequency of water application differ in the open field and greenhouse due to variations in evapotranspiration.

Three separate fields of approximately 400 m^2 were sampled for each management-cultivation method type in May, July and November, 2010 and February, 2011 at the same time of day. The fields were selected for their identical soil types and environmental conditions and differed only in the farm management and cultivation method used, *i.e.*, open field-conventional, greenhouse-conventional, open field-organic and greenhouse-organic. The treatment structure of the experiment was a repeated measures design with one repeated measures factor (season) and one non-repeated factor (farm management). One cropping season before the sampling started and during the sampling period, the crop rotations of all fields contained the same plant species to eliminate potential crop effects, and general management activities, such as planting dates, plowing and irrigation, were also controlled. Additionally, the soil was sampled prior to any management event (*e.g.*, plowing, fertilization or harvesting) to avoid soil disturbances influencing the soil properties. The samples were collected using a stainless steel corer (5.5 cm diameter) that was sterilized between uses, and each sample consisted of 7–10 random sampling points in each field from a depth of 0–20 cm at a distance of 10 cm from the crop row. The samples were thoroughly homogenized and sieved through 2 mm mesh to remove plant matter and earthworms, and the fresh soil was stored at −80 °C prior to DNA–RNA extraction.

2.2. Soil chemical and biological analysis

Soil moisture content was determined by drying the soils at 120 °C for 48 h. Microbial biomass C and N were determined in field-moist soil samples using a rapid chloroform-fumigation extraction method (Witt et al., 2000). Total soluble N was extracted from the field-moist samples using 2 M KCl (1:5, *w/v*). Total soluble N was measured with the alkaline persulfate oxidation method (Cabrera and Beare, 1993), and the nitrate (*i.e.*, nitrate and nitrite; nitrite concentration <1 mg kg^{-1} soil) and ammonium concentrations were determined with an automated flow injection analyzer (Smartchem 200; Westco, Frepillon, France). Soluble organic N (SON) was calculated as the difference between total soluble N and inorganic nitrogen (nitrate and ammonium). Soil pH was measured in 1:2 (*w/v*) air-dried soil:water extracts using standard electrodes (Mettler Toledo Delta 320; Mettler-Toledo, Greifensee, Switzerland). The EC of 1:1 air-dried soil:water extracts was measured using an EC meter (Ecoscan Con 6; Eutech Instruments, Singapore). Total C was measured using dichromate redox titration of air-dried soil that was ground and sieved to 0.5 mm (Skjemstad and Baldock, 2007).

2.3. Nucleic acid extraction and cDNA synthesis

Total DNA was extracted from 0.5 g (wet weight) of homogenized soil using the E.Z.N.A.™ Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions.