Pedobiologia 55 (2012) 271–281

Contents lists available at SciVerse ScienceDirect

Pedobiologia - International Journal of Soil Biology

journal homepage: www.elsevier.de/pedobi

Soil structural responses to alterations in soil microbiota induced by the dilution method and mycorrhizal fungal inoculation

Sarah L. Martin, Sacha J. Mooney, Matthew J. Dickinson, Helen M. West*

School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leicestershire LE12 5RD, UK

ARTICLE INFO

Article history:
Received 3 April 2012
Received in revised form 10 June 2012
Accepted 11 June 2012

Keywords:
Soil dilution method
X-ray Computed Tomography (CT)
Soil porosity
Aggregate stability
Arbuscular mycorrhizas

ABSTRACT

This investigation examines the effect of manipulating soil microbial community composition and species richness on the development of soil structure over a seven month period in planted (with or without mycorrhizal fungi) and in unplanted macrocosms. The dilution method effectively resulted in soil communities with consistently contrasting levels of species (TRF) richness. In particular, the 10^{-6} dilution of field soil resulted in less rich communities in bare unplanted soil than did the 10^{-1} soil dilution. However, this was not the case in planted soils where root activity was a powerful influence on species richness. After seven months, principal components analysis (PCA) separated bacterial community composition primarily on planting regime; planted mycorrhizal, planted non-mycorrhizal and bare soil treatments all contained different bacterial community compositions. A consistent finding in planted and unplanted soils was that aggregate stability was positively correlated with small pore sizes. Mycorrhizal colonisation decreased plant biomass and also resulted in reduced soil bacterial species richness, lower percentage organic matter and smaller pore sizes relative to planted but non-mycorrhizal soils. However, soil aggregate stability and water repellency were increased in these (mycorrhizal) soils probably due to AMF hyphal activities including enmeshment and/or glomalin production. In contrast, bacterial TRF richness was positively correlated with aggregate stability in the bare and non-mycorrhizal planted soils. Soil organic carbon was an important factor in all treatments, but in the bare soil where there was no additional input of labile C from roots, the percentage C could be directly related to fungal TRF richness. The less species rich bare soil contained more organic C than the more species rich bare soil. This suggests a degree of redundancy with regard to mineralisation of organic matter when additional, more utilisable C sources are unavailable. Understanding the effects of microbial diversity on functional parameters is important for advancing sustainable soil management techniques, but it is clear that soil is a dynamic ecosystem.

Introduction

It is widely acknowledged that soil systems are extremely diverse and complex (Giller et al. 1997; Torsvik and Øvreås 2002; Fitter 2005). Estimates of numbers of bacteria inhabiting soil range from 10^4 to 10^6 species in one gram of soil (Torsvik et al. 1990; Gans et al. 2005). Soil micro-organisms are vital for plant growth, nutrient cycling, decomposition and soil quality, yet relatively little is known about the role of biodiversity in this context. Characteristics of soil structure such as aggregation develop as a result of numerous factors including wet-dry cycles, clay flocculation, root activity, burrowing by soil organisms, fungal hyphal activity and microbial exudation (Tisdall and Oades 1982; Dexter 1988; Kleinfelder et al. 1992; Czarnes et al. 2000; Bossuyt et al. 2001; Denef et al. 2002; Scullion et al. 2002). Tisdall and Oades (1982) stated the importance of bacteria, fungi and roots as binding and stabilising agents within the soil environment, with their temporal contribution ranging from weeks to years. Feeney et al. (2006) suggested that soil structure and water repellency can be influenced by root and microbial activity extremely quickly. Their investigation showed that the number of aggregates of >2000 μm and their water repellency both significantly increased over a 30 day period; this was attributed to increased fungal activity, particularly in the rhizosphere. These authors used X-ray micro-Computed Tomography (μCT) to show that micro-organisms have an impact on development of soil structure and in particular on pore size distribution within aggregates. It is widely acknowledged that soil microbes significantly contribute to many soil ecosystem functions. What is not known however is how microbially diverse the soil ecosystem needs to be in order to maintain such functions. Relatively few experiments have attempted to differentiate between interacting organisms when considering the relative importance of biota on

* Corresponding author. Tel.: +44 115 9516268; fax: +44 115 9516261.
E-mail address: helen.west@nottingham.ac.uk (H.M. West).

0031-4056/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.pedobi.2012.06.001

soil structure (Hallett et al. 2009). Investigations that have concentrated on microbial populations have either been field studies focussing on reclamation or intensification gradients (Gomez et al., 2004), or relatively short-term laboratory culture studies (Franklin and Mills 2006). The dilution method of modifying microbial diversity has been frequently used in mineral soils (Griffiths et al. 2001; Wertz et al. 2006, 2007), peat (Dimitriu et al. 2010) and sewage (Franklin and Mills 2006). It is primarily used as a means of lowering species richness so functional ability can be correlated with biodiversity. Rarely is the function studied in this context related to soil porosity and the development of soil structure.

This investigation aimed to measure the relationship between microbial community structure and soil physical properties such as aggregate stability, pore size and pore distribution. Macrocosms of sieved sterile soil were inoculated with one of two dilutions of field soil to create microbial communities differing in species richness. Additional treatments included planting with *Plantago lanceolata* (± arbuscular mycorrhizal inoculum) or leaving the soil unplanted. The soil pore architecture was quantified from images derived by X-ray Computed Tomography (CT) and Terminal-Restriction Fragment Length Polymorphism (T-RFLP) analysis was used to characterise the microbial communities. Although roots and mycorrhizal fungi influence soil structure through their activity (Tisdall and Oades 1982; Angers and Caron 1998; Czarnes et al. 2000; Read et al. 2003), the relative importance of bacterial and saprotrophic fungal diversity in the development and maintenance of soil structure, has yet to be fully explored.

Materials and methods

Macrocosm setup

Sandy loam soil (Dunnington Heath series) was collected from 5 to 20 cm depth from the University of Nottingham farm site at Sutton Bonington, Leicestershire, UK (SK 512 267). The soil had the following physical characteristics: Sand 66%, silt 18%, clay 16%, organic matter 3.7% and pH 7.35. Soil was air dried and sieved to <2 mm before γ-irradiating at 25 kGy (Isotron Ltd, Daventry, UK). Sterilised soil was packed into macrocosms (7.4 cm internal diameter, 15.5 cm high, with a 400 μm mesh base) to a bulk density of 1.1 g cm^{-3}. Mycorrhizal treatments were inoculated with 6 g of crude arbuscular mycorrhizal fungal (AMF) inoculum consisting of root material, spores and an expanded clay carrier placed 5 cm beneath the soil surface. The inoculum was added as a layer rather than mixed homogeneously into the potting soil primarily to prevent it from directly affecting the structure of the soil and to allow it to be readily identified when the columns were imaged. Further, seedling roots had to penetrate the layer and this maximised initial contact with the inoculum. The inoculum contained five different *Glomus* species in combination (*G. intraradices*, *G. microaggregatum*, *G. mosseae*, *G. geosporum* and *G. claroides*) (PlantWorks Ltd, Sittingbourne, Kent, UK). Non-mycorrhizal (NM) treatments consisted of sterilised inoculum and sieved unsterilised washings. Columns were inoculated with indigenous micro-organisms originating from the fresh field soil, applied as one of two dilutions (Salonius 1981; Griffiths et al. 2001). Soil was serially diluted in sterile Ringer's solution (Dickinson et al. 1975) starting from a 10^{-1} (1:10) dilution up to 10^{-6}. Half the columns received the 10^{-1} dilution and the other half were treated with the 10^{-6} dilution; columns were initially saturated with the appropriate solution and then drained to field capacity. The experimental design was a factorial setup with further treatments superimposed onto each dilution amendment as follows: (i) bare soil, (ii) planted with *P. lanceolata* pre-germinated seedlings (at 1 true-leaf stage) + sterilised mycorrhizal inoculum, (iii) planted with *P. lanceolata* seedlings + live mycorrhizal inoculum. Two replicate columns were used for repeated non-destructive assessment of soil structure at 1, 3, 5 and 7 months from transplanting seedlings, using X-ray CT. A further three replicate columns were destructively harvested per treatment after one and three months, and four replicate columns harvested after five and seven months to give a total of 84 harvested columns. Columns were maintained in a glasshouse at 20 °C (± 5 °C) with supplementary lighting to give a 16-h day. Soil columns were maintained at field capacity by watering with sterile (autoclaved) deionised water; the quantity added was determined by weight. At each destructive harvest, a series of analyses were undertaken as described below.

Plant and mycorrhizal measurements

At each destructive harvest, root and shoot biomass were measured following oven drying at 80 °C until constant weight. Prior to drying, sub-samples of roots were weighed, cleared in 10% KOH and after rinsing in water, stained using 0.1% Chlorazol Black E lactoglycerol solution containing equal volumes of 80% lactic acid, glycerol and deionised water (Brundrett et al. 1984). After staining, the roots were transferred into glycerol for destaining and storage. Colonisation was quantified according to McGonigle et al. (1990) at ×200 magnification and data expressed as per cent root length colonised.

Quantifying soil biomass

After the root systems had been removed, the soil was homogenised gently prior to sub-sampling for immediate determination of soil moisture and organic matter content (loss on ignition). Additional 25 g sub-samples were analysed for microbial biomass-C using the fumigation-extraction method described by Vance et al. (1987) and quantified using a correction factor of 0.45 (Wu et al. 1990).

Characterisation of microbial communities using T-RFLP analysis

DNA was extracted from the soil using a PowerSoil DNA kit (Mo-Bio Laboratories Inc., Carlsbad, CA, USA) since this particular kit enables DNA cleaning. DNA extracted from the soil was amplified in the ITS-2 region for fungi and the 23S ribosomal subunit for bacteria. The fungal primers for amplification of the ITS-2 region were 5.8Sfor (5′-GCA TCG ATG AAG AAC GCA GC-3′) and FITSrev (5′-dyeD3 ATA TGC TTA AGT TCA GCG GGT-3′), labelled with the green WellRED dyeD3 (Sigma–Proligo, Gillingham, UK). The bacterial primers for amplification of the 23S ribosomal subunit (Anthony et al. 2000) were 23S for (5′-GCG ATT TCY GAA YGG GGR AAC CC-3′) and the reverse primer (23Srev) (5′-dyeD4 TTC GCC TTT CCC TCA CGG TAC T-3′), labelled with the blue WellRED dyeD4 (Sigma–Proligo, Gillingham, UK). Bacterial and fungal restriction digests were undertaken using the restriction enzyme *Hae*III and buffer 2 (New England BioLabs, Hitchin, Hertfordshire, UK) for fungal samples and enzyme *Mse*I and buffer C (Promega, Southampton, UK) for bacterial samples prior to analyses on a CEQ 8000 DNA analysis system (Beckman Coulter Inc., High Wycombe, UK). The relative abundance of each peak occurring (within each sample) at a dye signal greater than 100 was included in assessment, as this ruled out any background signal interference, with any shoulder peaks (associated with base pair addition through the use of PCR amplification) removed from analysis by grouping fragments with a band width of 1.25 bp (Edel-Hermann et al. 2004; Hodgetts et al. 2007). Bacterial TRFs selected for analysis ranged from 104 bp to 488 bp and fungal TRFs from 62 to 495 bp.