Soil Biology & Biochemistry 87 (2015) 110–121

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Changes in soil aggregation and microbial community structure control carbon sequestration after afforestation of semiarid shrublands

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ARTICLE INFO

Article history:
Received 27 October 2014
Received in revised form
27 March 2015
Accepted 24 April 2015
Available online 11 May 2015

Keywords:
Microaggregates within macroaggregates
Soil C pools
Organic amendments
Microbial activity
Basal respiration
Priming effect

ABSTRACT

Changes in plant cover after afforestation induce variations in litter inputs and soil microbial community structure and activity, which may promote the accrual and physical-chemical protection of soil organic carbon (SOC) within soil aggregates. In a long-term experiment (20 years) we have studied the effects, on soil aggregation and SOC stabilization, of two afforestation techniques: a) amended terraces with organic refuse (AT), and b) terraces without organic amendment (T). We used the adjacent shrubland (S) as control. Twenty years after stand establishment, aggregate distribution (including microaggregates within larger aggregates), sensitive and slow organic carbon (OC) fractions, basal respiration in macroaggregates, and microbial community structure were measured. The main changes occurred in the top layer (0–5 cm), where: i) both the sensitive and slow OC fractions were increased in AT compared to S and T, ii) the percentage and OC content of microaggregates within macroaggregates (Mm) were higher in AT than in S and T, iii) basal respiration in macroaggregates was also higher in AT, and iv) significant changes in the fungal (rather than bacterial) community structure were observed in the afforested soils (AT and T) – compared to the shrubland soil. These results suggest that the increase in OC pools linked to the changes in microbial activity and fungal community structure, after afforestation, promoted the formation of macroaggregates – which acted as the nucleus for the formation and stabilization of OC-enriched microaggregates.

1. Introduction

Among the ecosystem services provided by soils, climate change mitigation through C sequestration is of growing interest. This arises especially from the suggested limitations of emissions, based on a C credit trading system, in the Kyoto Protocol (Intergovernmental Panel on Climate Change, 1997; Six et al., 2002). Soil organic carbon (SOC) sequestration may be achieved by means of afforestation and other types of land-use conversion (De Gryze et al., 2004). Despite the considerable SOC sequestration potential of afforestation the results reported by different studies are contradictory (Wiesmeier et al., 2009; Cao et al., 2010; Laganière et al., 2010). This may be attributed to: (a) the environmental conditions, mainly rainfall regimes, and (b) according to the case of afforestation (to introduce trees for the first time in the area) or reforestation (to re-plant a formerly wooded area). A better understanding is needed of the mechanisms and factors controlling the accrual and stabilization of SOC following afforestation.

The amount and quality of plant litter inputs is a key factor controlling the accumulation of SOC (Kögel-Knabner, 2002), while promoting the processes involved in soil aggregation (Abiven et al., 2007). Physical soil properties such as soil structure or aggregation regulate many biological and chemical soil processes linked with C sequestration. Particularly, the formation of soil aggregates promotes the protection of organic matter against decomposition and oxidation (Jastrow et al., 2007). According to the conceptual model of Golchin et al. (1994), the fresh and labile pools of organic matter cause a rapid stimulation of the soil microbiota, accompanied by a significant increase in macroaggregates formation. Other authors showed significant correlations between the labile C pools and soil aggregation (Bhattacharyya et al., 2012). In addition, the O-alkyl groups – such as those of carbohydrates – have been considered as

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http://dx.doi.org/10.1016/j.soilbio.2015.04.012
0038-0717/

a major source of labile organic C for microbial activity, fostering the binding of clay and silt-size particles and the formation of microaggregates within macroaggregates, increasing the stability of soil aggregates (Jastrow, 1996; Six et al., 2000a). In addition to the substantial role of labile organic matter inputs, many studies have pointed out the important function of soil microorganisms in the formation and stabilization of soil aggregates (Díaz et al., 1994; Siddiky et al., 2012). The microorganisms act in two ways: a) fungal hyphae favor the mechanical union of soil particles and b) the exudation of byproducts promotes the coalescence of primary particles (De Gryze et al., 2005; Helfrich et al., 2008). Generally, fungi are thought to be more important in soil aggregate formation than bacteria (De Gryze et al., 2005). This has led to the suggestion that manipulations to enhance C sequestration should include shifting the soil microbial community towards an increased fungal component (Jastrow et al., 2007). In this sense, the change of microbial structure or the introduction of microorganisms into the soil, with lasting effects, is very difficult to tackle with current technologies (Jastrow et al., 2007). A possible option could be to cause changes in the vegetation cover through afforestation, since the vegetation type can influence the microbial community structure (Costa et al., 2006). Afforestation is a key land-use change across the world and is considered to be a dominant factor controlling ecosystems functioning and biodiversity; however, the response of soil microbial communities to this change is not well understood (Macdonald et al., 2009). Here, we intend to increase our knowledge of this response, by using next-generation sequencing techniques to provide a detailed analysis of the structure, diversity, and taxonomic composition of both the bacterial and fungal communities in natural shrubland and afforested soil under semiarid conditions.

In a previous publication, from the same experimental area, Garcia-Franco et al. (2014) showed that, based on results obtained 20 years after the plantation of trees, the afforestation of semiarid shrublands may result in either sequestration or loss of organic C in the ecosystem depending on the site preparation technique used. So, after 20 years the afforestation with soil organic amendment led to an increase of 1.3 kg C m^{-2} in the ecosystem, while without soil amendment a decrease of 0.6 kg C m^{-2} occurred. Here, we investigate the mechanisms of the processes defining the accrual and stabilization of SOC in afforested semiarid soils. Based on the earlier results, we hypothesized that the plantation of *Pinus halepensis* would increase fresh litter inputs into the soil, leading to (1) changes in soil organic C fractions which can related to soil organic C pools with different turnover rates, (2) changes in soil aggregate-size distribution due to the formation of new macroaggregates and organic C enriched microaggregates within macroaggregates, (3) increase in basal respiration within the macroaggregates as an indicator of higher microbial activity, which might be related with organic C protection in microaggregates formed within macroaggregates, (4) changes in the microbial populations structure due to the increase in ectomycorrhizal fungi associated with *P. halepensis*, which can produce aggregate-stabilizing mycelia, and (5) a close correlation between these changes in soil aggregation and microbial structure and activity. In our hypothesis we are assuming: (a) the OC accrual in microaggregates within macroaggregates is considered as an indicator of soil C stabilization and long-term sequestration (Six et al., 2013), and (b) the separated soil organic C fractions, arising from the fractionation procedure used, correspond to the sensitive and slow pools of Roth C (Zimmermann et al., 2007).

This long-term experiment was performed under environmental conditions typical of Mediterranean semiarid areas, so the results could be extrapolated to extensive areas of land around the world. The specific objectives of this study were to analyze the effects of the afforestation of degraded shrublands on: 1) changes in soil aggregation, 2) changes in the soil microbial community structure, and 3) physical–chemical processes of SOC protection and stabilization.

2. Material and methods

2.1. Site description and experimental design

The study area was located in the Sierra de Carrascoy (Murcia), Southeast Spain (37° 53′N, 1° 15′W, 180 m a.s.l). The climate is semiarid, with an average annual precipitation of 300 mm and a mean annual temperature of 18 °C. The mean annual potential evapotranspiration is 900–1000 mm y^{-1}. The soils are classified as *Haplic Calcaric Leptosol* with inclusions of *Haplic Calcisols and Leptic Calcisols* (FAO, 2006). The lithology is constituted by hard and compact limestone rocks. The fertility of the soils after each treatment is showed in Table 1. The dominant vegetation is composed of species typical of Mediterranean shrublands, such as *Rosmarinus officinalis* L., *Thymus vulgaris* L., and *Anthyllis cytisoides* L. with scattered *P. halepensis.* Miller.

The experiment site was established in October 1992 in an area of 1800 m^2 and consisted of three 20 m × 30 m plots located on an east-facing hillside (25% mean slope), to test the following afforestation techniques: a) mechanical terracing with a single application of 10 kg m^{-2} of an organic amendment, which consisted of the organic waste of urban soil refuse (USR) (García et al., 1998), and *P. halepensis* plantation (plot AT), and b) mechanical terracing and *P. halepensis* plantation, without organic amendment (plot T) addition. To test these afforestation techniques, an adjacent Mediterranean shrubland was considered as the control plot (S). More details about these afforestation techniques are given in Garcia-Franco et al. (2014).

2.2. Soil sampling design

In April 2012, 20 years after afforestation, a randomized soil sampling trial was designed to assess the effects of the tested factors. Six (1 m × 1 m) soil sampling sub-plots were selected at each plot (18 sampling sites). The separation between sampling sites was about 10 m in one direction and 15 m in the other. The sampling sites were located under trees in treatments AT and T and under shrubs in the control S. At each sampling site, soil samples were collected from three soil depths: 0–5 cm, 5–20 cm, and 20–25 cm

Table 1
Soil properties of the topsoil (0–5 cm depth) in AT (afforested + organic amendment), T (afforested), and S (shrubland).

Soil properties	Treatments		
	S	T	AT
Organic carbon (g kg^{-1})	12.8 ± 0.7a	12.5 ± 0.7a	22.6 ± 2.5b
Total N (%)	0.19 ± 0.01ab	0.15 ± 0.02a	0.23 ± 0.02b
Available P (mg kg^{-1})	8.9 ± 0.1b	4.8 ± 0.1a	24.9 ± 1.0c
Available K (meq 100 g^{-1} soil)	0.72 ± 0.03c	0.39 ± 0.01a	0.57 ± 0.02b
pH	8.1 ± 0.18a	8.0 ± 0.1a	7.9 ± 0.1a
Carbonates (%)	29.5 ± 1.1a	41.4 ± 1.5b	47.2 ± 1.3b
Bulk density (g cm^{-3})	1.13 ± 0.06a	1.26 ± 0.01a	0.84 ± 0.19b
Water holding capacity (%)			
Field capacity (−33 kPa)	21.9 ± 1.4b	16.2 ± 0.6a	22.0 ± 1.0b
Permanent wilting point (−1500 kPa)	10.5 ± 1.2a	10.8 ± 0.7a	12.7 ± 0.6[a]
Available water content (%)	11.5 ± 1.6b	5.4 ± 0.5a	9.3 ± 0.5b
Texture	Loam	Loam	Silt loam

Numerical values are means ± standard errors for n = 6. Different letters in rows indicate significant differences between treatments (Tukey's test, $P < 0.05$).