



Immediate and long-term effect of tannins on the stabilization of soil aggregates



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ABSTRACT

Soil aggregates are organomineral associations with a fundamental importance for soil structure and function. Litter from vegetation alters aggregate formation and stability, and polyphenols such as tannins found in leaves, roots and wood play an important role in soil biogeochemical and biological processes. However, the effect of tannins on soil physical properties remains largely unexplored. We hypothesized that tannins influence aggregate stability through their ability to (i) complex proteins in the soil and (ii) perturb the gelling property of root mucilage. Therefore, Mediterranean soil aggregates were incubated with condensed tannins, either as a pure substrate or in combination with a standard protein (bovine serum albumin, BSA) and with a model root mucilage polysaccharide (polygalacturonic acid, PGA) able to form gel-like structures with divalent cations (notably Ca^{2+}) widely present in Mediterranean calcareous soils. The changes in aggregate stability were monitored under controlled conditions, immediately after the addition of tannins, after 2 weeks, 3 and 6 months of incubation. Tannins added alone did not yield a significant effect on aggregate stability. However, modulatory effects were found when combinations of treatments occurred. Tannins positively modulated the stabilizing effect of the BSA, giving credit to our hypothesis on the stabilizing role of the tannin-protein complex forming macromolecules, thus enforcing soil particle cohesion within aggregates. However, tannins negatively altered the stabilizing effect of PGA, suggesting that the expected perturbation of the PGA gelation occurred, with detrimental consequences for aggregate stability. Over time, tannins maintained the effect of BSA, suggesting a protective effect of tannins, possibly linked to their ability to slow down the degradation of nitrogen compounds through protein binding. Overall, we showed that tannins reacted with other organic compounds resulting in specific effects on physical soil properties, thus demonstrating that tannins in soils play a role beyond their effects on biogeochemical aspects.

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1. Introduction

Aggregate stability is a key indicator of several soil properties, such as erodibility (Barthès and Roose, 2002; Ding and Zhang, 2016), carbon storage (Jastrow and Miller, 1997) or penetration by roots (Angers and Caron, 1998). Soil organic matter has been widely recognized as a major determinant of aggregate stability (Abiven et al., 2009; Le Bissonnais et al., 2007; Six et al., 2004). The

influence of organic inputs depends on their nature (Abiven et al., 2009; Monnier, 1965). Certain specific organic compounds have been studied in detail, such as root and microbial polysaccharides (Chenu, 1989; Chenu et al., 1994; Schlecht-Pietsch et al., 1994; Traoré et al., 2000; Czarnes et al., 2000; Zibilske et al., 2000) or fungal-related proteins (Rillig, 2004; Wright and Upadhyaya, 1998), which are shown to have an important, positive, short to mid-term stabilizing role. In contrast, other specific compounds such as plant tannins, which are polyphenols of high molecular weight, have been poorly explored. To our knowledge, the only study on tannins showed that tannic acids prolonged the effect of polysaccharides

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(Griffiths and Burns, 1972). Nevertheless, tannins can be considered as good candidates for stabilizing soil aggregates for different reasons: (i) large amounts of these compounds can enter the soil from leaf and root litter (Gallet and Lebreton, 1995; Hättenschwiler and Vitousek, 2000; Kraus et al., 2003; Meier et al., 2008); (ii) tannins possess a rapid sorption ability and remain for a longer time in soils than other substances (Schmidt et al., 2012), especially condensed tannins (Nierop and Verstraten, 2006), and most importantly (iii) tannins are known to react with different classes of macromolecules, which influence aggregate stability. In particular, the reaction of tannins with proteins is known to occur through multiple hydrogen bonds and hydrophobic interactions, resulting in the formation of macromolecular cross-linked complexes (Hagermann, 1989; Hättenschwiler and Vitousek, 2000; Kraus et al., 2003). This ability of tannins to form complexes suggests a potentially important role in the stabilization of soil aggregates, through enhanced chemical bonding between organic soil compounds, and in particular proteins already present in the soil. When in contact with polysaccharides, tannins are known to perturb the gelling process of these macromolecules because of their function as proton scavengers (Wüstenberg, 2014). Therefore, tannins could also inhibit the gelling process of certain polysaccharides found in root mucilage. Polysaccharides in root mucilage react with calcium (Ca^{2+}) to form gel-like structures (de Kerchove and Elimelech, 2007), with a strong positive effect on soil stability (Czarnes et al., 2000). If tannins alter mucilage properties, they will have a potentially negative effect on soil aggregate stability.

Mediterranean road embankments provide a useful terrain for exploring the links between tannins and soil aggregate stability. Sparsely covered by plants after road construction, vegetation cover of embankments then follows a successional trend with significant changes in plant community composition (Odum, 1969), resulting in the replacement of grass and herb species (generally with low tannin concentrations in leaves and roots), by small shrubs and tree species (with higher quantities of tannins). In the Mediterranean region, concentrations of tannins in plant roots were found to range from 0.2 ± 0.2 to $9.42 \pm 4.4\%$ of tannins per gram of dried roots between early (>10 years-old) and late successional plant communities (40–69 years-old) (Balmot, unpublished data). These differences in tannin concentrations should therefore result in an increase of tannins in litter and soils along a successional gradient (Nierop and Verstraten, 2006), but whether they play a role in the stabilization of soil aggregates, which also augments along successional gradients (Andres and Jorba, 2000; Erktan et al., 2016), is not known.

We explored the role of tannins in the stabilization of Mediterranean soil aggregates from road embankments and tested whether this effect varied over time. We hypothesize that tannins influence soil aggregate stability through: (i) their ability to complex proteins and (ii) their ability to perturb the stabilizing gelling property of the root mucilage analogue polygalacturonic acid (PGA). If, according to our first hypothesis, tannins enhance aggregate stability by binding proteins and other macromolecules already present in soil, then soil enrichment with proteins prior to tannin addition should result in an increased positive impact of tannins. Similarly, if, as in our second hypothesis, tannins decrease aggregate stability by perturbing the gelling of the PGA, then soil enrichment with PGA prior to tannin addition should enhance such negative modulation.

To test these hypotheses, we observed the impact of tannins on the aggregate stability of two soils from road embankments, representing early and late plant successional stages. Experiments were conducted under controlled conditions, and tannins were added either alone or in combination with protein or polysaccharide compounds.

2. Material and methods

2.1. Site selection, soil sampling and isolation of soil aggregates

Soil samples were collected from road embankments near Montpellier in Southern France. The area is characterized by a Mediterranean sub-humid climate, with frequent freeze and thaw cycles in winter and a warm, dry summer. Over a 20 year period (1996–2015), mean annual temperature was 14.6 ± 6.8 °C with major daily variations (12.4 ± 4.7 °C) (INRA, Agroclim, France). Over the same period of time, mean annual precipitation was 774 ± 210 mm with most rainfall occurring in the autumn (3.9 ± 8.7 mm as daily precipitation average in September over the 1996–2015 period; INRA, Agroclim, France). Soils on road embankments are shallow anthropogenic soils that were highly disturbed during road construction and then left bare, and so are particularly prone to erosion. The origin of the soil material used for the construction of roadside embankments is not known, but most probably come from the surrounding area, characterized by brown calcareous soils (FAO classification Calcic Luvisol) formed on limestone bedrock. A few months after roadworks, hydro-seeding was performed with seed mixtures (composition not known). After several years, successional dynamics resulted in further changes in plant community composition (Bouchet, Unpublished data).

Two road embankments were selected for soil sampling, one undergoing an early successional stage (6 years old; $43^{\circ}42'58.1''\text{N}$; $3^{\circ}49'44.4''\text{E}$) and the other undergoing a later successional stage (69 years old; $43^{\circ}42'27.5''\text{N}$; $3^{\circ}47'56.3''\text{E}$; Fig. 1). In the early successional stage, the plant community was dominated by herbs, with a sparse plant cover, while in the late successional stage plant cover was denser with a higher proportion of small shrubs (Appendix S1). We assume that differences in plant species' composition resulted in contrasting tannin concentrations in the different communities. We quantified tannin concentrations, using the method Butanol HCl described in Porter et al. (1986) and Gessner and Steiner (2005), of mixtures of fine roots representative of communities on both embankments. We found that no tannins were detectable in the roots of the early successional community while 4.02% tannins per gram of dried roots were present in the late successional community.

On each road embankment, four soil samples (0–10 cm depth) were collected (homogeneously distributed across the site; Fig. 1) and pooled together to determine the following soil properties (Table 1): clay, silt, sand, soil organic carbon (SOC), total soil nitrogen (N), carbon : nitrogen (C:N) ratio and pH (in water). The soils from the two selected sites both possessed a dominant silty texture and high carbonate concentrations, with a higher clay content in the early successional soils (Table 1). SOC was low in the early successional site and high SOC content was found at the late successional site (Table 1).

On each road embankment, three replicate soil samples (0–10 cm depth; 2–3 kg of soil) were extracted (Fig. 1) and used during the soil incubation experiment. Then, soils were air-dried for one week and soil aggregates (3–5 mm) were obtained by sieving. Finally, initial aggregate stability was measured for each of the three replicate soil samples.

2.2. Treatment design

We tested the impact of tannins, alone or in mixtures with other organic compounds, on soil aggregate stability (Fig. 2). A commercial mix of condensed tannins (VR Grape, Laffort) was used with a concentration of 1 mg mL^{-1} (eq. 0.167 g kg^{-1} soil) to mimic previously observed soil concentrations (Adamczyk et al., 2014). To amplify the hypothesized “binding” effect of tannins through

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