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# Arbuscular mycorrhizal fungal hyphae reduce soil erosion by surface water flow in a greenhouse experiment

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Abstract

The role of arbuscular mycorrhizal fungi (AMF) in resisting surface flow soil erosion has never been tested experimentally. We set up a full factorial greenhouse experiment using *Achillea millefolium* with treatments consisting of addition of AMF inoculum and non-microbial filtrate, non-AMF inoculum and microbial filtrate, AMF inoculum and microbial filtrate, and non-AMF inoculum and non-microbial filtrate (control) which were subjected to a constant shear stress in the form of surface water flow to quantify the soil detachment rate through time. We found that soil loss can be explained by the combined effect of roots and AMF extraradical hyphae and we could disentangle the unique effect of AMF hyphal length, which significantly reduced soil loss, highlighting their potential importance in riparian systems.

Keywords: Soil erosion, concentrated flow, soil detachment rate, AMF

The rate of soil loss by erosion has been accelerated due to various human activities at a global scale (Grimm *et al*., 2002), with negative effects including loss of topsoil, decrease in soil organic matter, and pollution of surface waters (Lal, 2001). Soil erosion is related to the susceptibility of soil to both detachment and transport of soil particles (Gyssels *et al*., 2005). Vegetation biomass, both above and belowground, has been identified to play a role in decreasing soil erosion (Prosser *et al*., 1995; Gyssels and Poesen, 2003). The role of soil biota has not often been subjected to empirical tests, but it is assumed that members of the soil biota indirectly decrease soil erosion through the formation and stabilization of soil aggregates (Tisdall and Oades, 1982; Rillig and Mummey, 2006). For example, arbuscular mycorrhizal fungi (AMF) are root associated fungi known for their role in increasing soil aggregation (Tisdall and Oades, 1982; Mardhiah *et al*., 2014;

Leifheit *et al*., 2014) through their extended extraradical hyphae in the rhizosphere (Tisdall and Oades, 1982; Rillig and Mummey, 2006) and by stimulating root growth (Bearden and Petersen, 2000).

In order to quantify the role of AMF hyphae in reducing soil erosion, we measured at the end of a greenhouse experiment the difference in soil detachment rate (g soil 10 $s^{-1}$) under a constant flow of water across a fixed area of soil surface (63.6 $cm^2$) at successive points in time, comparing different treatments (AMF treatment, microbial filtrate treatment, AMF and microbial filtrate treatment and control). *Achillea millefolium* seeds were surface sterilized in 70% ethanol and 5% commercial bleach. We added 5 seeds per pot and then thinned to two plants per pot. We used a sandy loam alluvial soil (73% sand, 18% silt and 7% clay (Rillig *et al*., 2010)), which was autoclaved twice (121°C, 20 minutes) and was re-mixed before placing into each pot (1.3 kg of soil per pot). Pots in AMF treatments received 150 *Glomus intraradices* (*Rhizophagus irregularis*) spores; non-AMF treatment pots received the same amount of sterile carrier material. We prepared the microbial filtrate, which might introduce saprobic fungi and bacteria, by passing a suspension of the soil used in the study (200 g $L^{-1}$) through a 20 μm size sieve and used the slurry as microbial filtrate treatment. Pots in microbial filtrate treatments received 2 ml of the slurry, while those in non-microbial filtrate treatment received the same amount of sterile slurry. The greenhouse temperature was 16-22°C and the experiment lasted for ~ 23 weeks. The plants were of similar size by the end of the experiment.

To measure the soil erosion due to water flowing over the soil surface, a hydraulic flume, 2 m in length and 0.1 m wide, was constructed using a transparent Plexi glass wall at the University of Trento, Italy. At 20 cm before the end of the flume, a hole with a 9 cm external diameter was created to hold the soil core. A sharpened PVC pipe (inner diameter = 9 cm), made to fit the flume hole, was used as a corer and was carefully placed at the centre of each of the pots and pushed through the soil from the top until it reached the bottom of each pot. The corer was then pushed through from below and towards the surface of the flume bottom using a piston so that the soil surface was maintained in line with the flume bed through each experiment (Suppl. Mat. Figure S1). The flume was set at a slope of 18°, and a flow of tap water was discharged into the flume at a constant rate (0.0003 $m^3 s^{-1}$). Mean flow velocity (1.17 ± 0.01 $m s^{-1}$) was measured every day and yielded a mean flow shear stress on the soil surface of 7.75 Pa (Suppl. Mat. Equation S1).

Ten replicate samples were prepared according to each treatment. Samples were prepared with methods adjusted from De Baets *et al*. (2006). The samples were retained within a constant water level environment (4.5 cm below the soil surface) to allow slow capillary rise and all aboveground biomass was clipped. The samples were drained immediately prior to being introduced to the flume, where they were subjected to a constant discharge for 145 seconds. Following an initial flow period of 20 seconds, samples of the water draining from the flume were taken every 15 seconds for 10 seconds, providing a total of five successive 10 second samples (R1-R5). The samples were left to settle before decanting the water, which was oven dried at 65°C and then the residue was weighed.

Soil which was left in the corer was carefully retained and dried. To ensure that measurements of the soil left in the corer did not include soil and roots exposed by the soil erosion experiment, we carefully scraped a thin layer of the surface layer off each cored soil. After sieving the soil through a 4-mm sieve, aggregate stability was measured by re-wetting 4.0 g of soil using capillary action and sieving for 5 minutes on a 250 μm sieve before drying at 65°C. The dried material was then crushed and passed through the sieve, separating the stable aggregates from the coarse fraction. Root biomass was extracted and measured using an extraction-flotation method (Cook *et al.,* 1988). Root length grouped by diameter (Barto *et al*., 2010) was measured by analyzing scanned images using WinRhizo Pro 2007d (Regent Instruments Inc., Quebec City, Canada). Hyphae were extracted from 4.0 grams of dried soil using a protocol adapted from Jakobsen *et al*. (1992) and then stained with Trypan Blue. AMF and non-AMF extraradical hyphal length were measured according to Rillig *et al*. (1999).

We used the Kruskal Wallis test to quantify the difference of soil detachment rate (g soil 10 $s^{-1}$) between treatments at each of the five successive time points during the flume experiments. We also ran linear models correlating total soil loss with soil detachment rate determinants (percent water stable aggregates (% WSA), root biomass, very fine, fine and coarse root length, AMF and non-AMF extraradical hyphal length) tested as main effect and interaction. We calculated variation in partitioning of root biomass and AMF extraradical hyphal length using redundancy analysis. All statistical analyses were conducted using version 2.14.0 of the R statistics software (R Development Core Team, 2012).

In general, soil loss decreased through time (Suppl. Mat. Figure S2). A possible explanation is that initially, relatively loose surface soil which came into contact with the erosion flow was rapidly detached; soil loss then slowed, possibly because of more intense effects of roots with or without fungal hyphae. We found that AMF treatments decreased soil loss most effectively compared to the control (Figure 1). Total soil loss can be explained by the joint effect of total root biomass (17%) and AMF extraradical hyphae (16%) (Table 1). AMF extraradical hyphal length significantly decreased total soil loss when used in linear models as a singular main effect and in interaction with root biomass (Suppl. Mat. Table S1, Figure 2). This is to our knowledge, the first time that AMF extraradical hyphal length has been shown to have a direct effect in reducing surface soil erosion due to surface flow. The role of AMF seems to be due to the ability of AMF to produce extraradical hyphae. The addition of microbial filtrate did not reduce the soil detachment rate compared to the control and even reduced the effectiveness of AMF treatment. We also did not find a significant difference of %WSA between treatments (Suppl. Mat. Table S3) and no significant correlations between the soil detachment rate and % WSA in our models (data not shown). This implies that soil aggregate stability in our system was not an important factor for preventing soil erosion due to concentrated flow. Studies showed that besides soil aggregates, microtopography (surface roughness) and soil cohesion due to a dense root mat, can decrease surface soil erosion (Campbell *et.al*., 1989; Prosser *et al*., 1995; Prosser and Dietrich, 1995; Hu *et al*., 2002). Our study implies that, rather than the role in formation or maintenance of stable soil aggregates, the role of AMF hyphae -which might also include the formation of a hyphal network which further increases soil cohesion- might be more important in reducing surface soil erosion. Although the microbial filtrate might contain saprobic fungi which also produced hyphae, their minimal effect towards reduced soil erosion in this study might imply that the hyphae of both fungal groups behave differently. AMF tend to produce more persistent, coarser and thicker extraradical hyphae compared to many saprobic fungal hyphae (Klironomos and Kendrick, 1996; Klironomos *et al*., 1999; Allen, 2006). Saprobic fungi can also produce enzymes degrading soil carbon, an ability which AMF lack; this taken together could explain the significant role of AMF in reducing soil erosion in our experiment. Overall, our results highlight the role of AMF in potentially stabilizing soils in riparian systems.

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Table 1. Variation partitioning based on redundancy analysis was used to explain the pattern of total soil loss in relation to explanatory variables: AMF extraradical hyphal length and root biomass. All percentages explained were significant (p-values < 0.05).

| ***Response variable:*** **Total soil loss (g soil in 50 s)** | df | Fraction explained (%) |
|---|---|---|
| *Explanatory variables:* | | |
| AMF extraradical hyphal length fraction (*with covariable:* root biomass) | 1 | 16 |
| Root biomass fraction (*with covariable*: AMF extraradical hyphal length) | 1 | 17 |
| Total | 2 | 28 |
| Shared fraction | 0 | 4.1 |
| Residuals | - | 76 |
| AMF extraradical hyphal length *(without covariable)* | 1 | 9.7 |
| Root biomass *(without covariable)* | 1 | 10.2 |

Figure captions

Figure 1. Linear models fitted using the generalized least squares (GLS) method corrected for heterogeneity of variances (var = varIdent(form=~1|fcategorical)) were used to plot cumulative soil detachment rate through time (R1, R2, R3, R4, R5) for different treatments ("control", "AMF treatment", "AMF and microbial filtrate treatment" and "microbial filtrate treatment"). Figure shows fitted lines with significant differences between each treatment levels (Suppl. Mat. Table S2). Different symbols indicate different treatments (control = Δ, AMF treatment = ●, AMF and microbial filtrate treatment = ○, microbial filtrate treatment = +). The highest data point (microbial filtrate treatment, ranging 12.15-30.03 g soil 10 $s^{-1}$, R1-R5) was omitted to enable clear visualization of data.

Figure 2. A linear model fitted using the generalized least square (GLS) method corrected for heterogeneity of variances (var = varIdent(form=~1|fcategorical)) and spatial autocorrelation was used to correlate total soil loss (y axis) to AMF extraradical hyphal length (x axis).