Soil Aggregation and Arbuscular Mycorrhizal Fungi as Indicators of Slope Rehabilitation in the São Francisco River Basin (Brazil)

Andrej C. KIMURA and Maria Rita SCOTTI

Department of Botany, Institute of Biological Science, Federal University of Minas Gerais, Belo Horizonte, Brazil

Abstract


Anthropogenic activity along the Velhas River (São Francisco River basin) has destabilized the banks of the river channel across an urban fragment. To improve the physical stabilization, the base of the slope was stabilized with urban construction waste. After this, the slope was revegetated with native species and arbuscular mycorrhiza fungi (AMF) inoculation was applied with a successfully restoration of the vegetative cover and ecological functions. This study aims to evaluate the role of the AMF population in the soil aggregation and stabilization of the revegetated slope. The soil aggregation was higher at the experimental site than at the disturbed site, especially under the AMF inoculation. The aggregates improvement was accompanied by an increase of soil humic acid and glomalin contents 24 months after the transplantation despite a flood impact 12 months after the transplantation. A scatter plot based on Principal Component Analysis of aggregates showed that the preserved site samples clustered with most of those from experimental site. However, some samples from the experimental site were found between those from preserved and disturbed sites. This result shows that the recovering site is evolving toward the conditions of the preserved site and that the rehabilitation process is in an intermediate phase related to the aggregate formation. The AMF inoculation of woody species was indicated in the rehabilitation procedures.

Keywords: glomalin; humic acids; landscape alteration; microbial biomass; river bank stabilization

The Velhas River is the principal tributary of the São Francisco River, and its channel is open and shallow. Therefore, its flow depends on the flooding of the riparian area in the rainy season. It is considered the most severely affected river of the São Francisco Basin due to several anthropogenic factors, such as inappropriate land use for agriculture, pasture, industrial and mining activities. Moreover, other factors, such as the flood proofing of urban tributary rivers and the high sewage load, have contributed to severe impacts on the Velhas River. Over time, all of these disturbances have altered the hydrology of the landscape, resulting in erosion and sedimentation. As a result, mass-wasting processes have destabilized the channel banks and widened the channel. In certain sections of the Velhas River, the height and angle of the bank can exceed the critical conditions for stabilization, resulting in shear deformation of the material and washouts at the base of the slope. When erosion reaches a level that produces a loss of resilience, resulting in destabilization and erosion, the ecosystem becomes maximally degraded (King & Hobbs 2006).

For stabilization against superficial erosion, especially in slope areas, vegetation appears to be the chief means of structural protection due to the role of plant roots (Reubens et al. 2007) by either hydrological or mechanical mechanisms (Nilaweera & Nutalaya 1999). Hydrological stabilization is related to the role of vegetation, facilitating evapotranspi-
ration and soil porosity, which, in turn, control soil infiltration. Mechanical stabilization refers to the anchoring effect of coarse and fine roots (Nilaweera & Nutalaya 1999).

However, the aggregation of soil is the fundamental property that determines resistance to erosion and degradation as well as productivity (Six et al. 2000). Soil water-stable aggregates (WSAs) are the best indicator of the ability of a soil to resist erosion (Barthè & Roose 2002; An et al. 2010). Aggregate stability is a highly complex parameter influencing a wide range of soil properties including carbon stabilization, soil porosity, water infiltration, aeration, compactability, water retention, hydraulic conductivity, resistance to erosion by water and overland flow (An et al. 2010). Therefore, aggregate stability has been considered an indicator of soil quality.

Aggregate formation and stabilization depend on the formation of soil organic matter (SOM). The principal basis of the aggregation process is the ability of humic acid polymers to bond different particles, and the clay–humic complex is the primary unit of aggregation (primarily due to humic acid) that improves aggregate stability (Piccolo & Mbagwu 1994). Humic acid interacts with clay and forms an organometal complex (Edwards & Bremner 1967) with microaggregates sizing < 250 µm. These stable microaggregates, in turn, are bound together into macroaggregates (> 250 µm) by the transient and temporary action of fungal hyphae and roots as well as by polysaccharides produced by microorganisms and plants. Macroaggregate stability is correlated with the relative content of humic substances (Šimanský & Bajčan 2014).

Arbuscular mycorrhizal fungi (AMF) have been considered an important instrument for the reclamation of stressed sites due to their significant role in plant establishment even in the early stages of revegetation. AMF also play an important role in soil aggregation (Rillig et al. 2010), not only through the physical action of their hyphae, but also due to their ability to produce heat-stable proteins, so called glomalin-related soil proteins (PRSG) (Wright & Upadhyaya 1996). PRSGs are small hydrophobic proteins found in the hyphae of many types of mycorrhizal fungi. These proteins contribute to the insolubility and hydrophobicity of aggregates (Rillig et al. 2003).

In particular, soil aggregate formation promoted by humic acids modifies the quantity and size of pores (macro- and microporosity), which ensures soil aeration and drainage (Stevenson 1994).

To physically stabilize the slope with a negative angle on the bank of the Velhas River, urban concrete construction wastes were deposited at the base of the slope 8 m deep in the river. The slope was revegetated after this physical intervention. Stability of soil aggregates is an appropriate parameter for predicting the potential risk of water erosion in this revegetated slope and as an indicator of soil erodibility (An et al. 2010). Besides, vegetation and mycorrhizal associations could modify qualitative and quantitative soil aggregate formation.

Therefore, this study aims to evaluate the soil aggregation of the revegetated area and the soil abiotic and biotic factors related to aggregation as indicators of the soil stability and success of the rehabilitation process.

**MATERIAL AND METHODS**

**Experimental site.** The experimental area is a slope (Figure 1) located on the left bank of the Velhas River in the city of Belo Horizonte, State of Minas Gerais, Brazil. This urban site belongs to the neighbourhood Beija-Flor (19°50'20.33"S, 53°51'59.13"W). The predominant vegetation is tropical savanna (Brazilian Cerrado). Annual temperature averages 22–23°C. Rainfall occurs primarily in summer, winters are dry, and the total annual rainfall is 1200 mm. The slope suffered a flood at 12 months after the transplantation. During the flood event in the São Francisco Basin the flow rate increased from 2810 to 8000 m³/s (Godim Filho et al. 2004).
A preserved riparian forest located in an Environmental Protection Park (19°52’47”–19°52’34”S, 44°07’44”–43°47’30”W) was chosen as a reference area or protected site near the experimental site (50 m), according to Stoddard et al. (2006). The native vegetation found in the preserved riparian forest is tropical savanna (Cerrado). The riparian area without vegetation adjacent to the base of the slope in the degraded section of the Velhas River was chosen as the control site.

**Experimental design.** After the physical stabilization of the linear slope with urban construction wastes (material from demolition of urban buildings), the slope showed an angle of approximately 45° (performed by Eco Maquinas Company, Belo Horizonte, Brazil) (Figures 2A and 2B) and it was revegetated (Figure 2C). The flood reached the experimental site 12 months after the transplantation (Figure 2D). The experiment was conducted as a randomized block design, with an area of 0.2160 ha (135 × 16 m = 360 m²), with a 1 m border on each side (Figure 3).

**Planting.** The area was planted with seedlings of shrub and tree species: *Psidium guajava, Eugenia uniflora, Croton urucurana, Morus nigra, Inga edulis, Erythrina speciosa, Jacaranda mimosifolia, Hymenaea courbaril, Piptadenia gonoacantha, Samanea inopinata,* and *M i-

![Figure 2](image2.png)

Figure 2. Slope rehabilitation: urban concrete construction wastes were used as fill at the base of the slope (A), slope before revegetation (B), slope 12 months after revegetation (C) and slope under flooding (D)

![Figure 3](image3.png)

Figure 3. Experimental design of slope revegetation: inoculated plots with arbuscular mycorrhizal fungi (1), uninoculated plots (2)
*mosa bimucronata.* After four months of growth under nursery conditions, the seedlings were transplanted to the field. The inoculation of 50% of the species was performed under nursery conditions. A spacing of 3 × 3 m was maintained, and fertilization was performed according to Somasegaran and Hober (1985). After transplantation of the native woody species, seeds of commercial herbaceous species were planted in-between the rows for surface erosion control.

**Inoculants.** The shrubs and trees were inoculated with the spores of AMF of the species *Gigaspora margarita,* *Acaulospora scrobiculata,* and *Glomus etunicatum* from the collection of the Laboratory of Interaction of microorganisms and plants. The plants were grown in the greenhouse of the Biological Science Institute of the Federal University of Minas Gerais (ICB-UFMG) and inoculated in a plant nursery. A total of 1 ml suspension of 50 spores of each AMF type (150 spores per pot) was used for inoculation. Commercial herbaceous seeds were inoculated using a mixture of seeds with soil containing spores of the above-mentioned species (220 spores/100 g soil) before sowing in the soil. This mixture was sown in the soil in-between the rows where the woody species were planted. Therefore, inoculated plots received both plants and seeds which were inoculated with AMF.

**Plant growth and establishment.** The soil coverage was estimated using a 1 m² quadrant that was subdivided into 100 identical cells of 10 × 10 cm (Toledo & Schultze-Kraft 1982), which were placed in each plot. The coverage in each cell was recorded. Three quadrant samples were collected per plot or treatment per block at 24 months after transplantation (3 replicates × 2 treatments × 3 blocks), and the plant presence per quadrant was estimated (%).

**Soil sampling.** The samples were collected from 0–20 cm depth at 6 and 24 months after planting. From each plot (360 m²), 6 soil samples were collected. There were totally 18 samples per treatment/time or 36 samples in total (6 soil samples × 2 treatments × 3 blocks) which underwent all physical, chemical, and biological analyses.

Similar sampling was performed in the adjacent preserved area (the preserved riparian forest used as the reference site) and at the disturbed site on the Velhas River.

**Soil analysis**

**Textural and soil fertility analyses.** Soil samples were sieved through a 2 mm mesh and analyzed for physical-chemical properties at 6 and 24 months after planting (EMBRAPA 1997). Besides textural analysis, the experimental site was compared with the preserved site in relation to the cation content, cation exchange capacity, base saturation, organic matter content, soil pH, among other parameters, using Tukey’s Multiple Range test at the 5% confidence level ($P \leq 0.05$).

**Determination of soil aggregation and porosity.** The analyses were performed on soil samples sieved at 2 mm. Soil aggregation measurements were carried out to measure the proportion of water-stable aggregates (WSAs), using standard methods (Kemper & Rosenau 1986). Undisturbed soil samples from the studied sites were collected in cylinders of a predetermined volume and sent for porosity analysis according to the Brazilian Agricultural Research Corporation (EMBRAPA 1997).

**Microbial biomass (Cₘₑᵦ) and soil population of AMF and glomalin content.** The Cₘₑᵦ analysis was performed with the fumigation-extraction method according to Vance *et al.* (1987) using fumigated (CCl₄) and non-fumigated soil samples. The mycorrhizal spores were recovered from the soil by the sieving and decanting method according to Gerdemann and Nicolson (1963), and the data were expressed as the number of spores/1 g dry soil. Healthy spores were counted. Each spore type was mounted sequentially in polyvinyl-lacto-glycerol (PVLG) and Melzer’s reagent (Morton 1988) for identification according to the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM; http://invam.caf.wvu.edu). Glomalin-related proteins (PRSG-T) were extracted using the method of Wright and Upadhyaya (Wright & Upadhyaya 1996, 1998), and the protein content was estimated by the Bradford method (Bradford 1976).

**Fractionation of soil organic matter.** The fractionation of sequestered carbon from soil organic matter was performed according to Dabin (1971) using 15 g of sieved soil per treatment per block to obtain the fraction of carbon represented by humic acid and fulvic acid.

**Statistical analysis.** The results of the samples with normal distribution were subjected to one-way ANOVA using Minitab software (Version 13.2), and the means of the treatments were compared using Tukey’s Multiple Range test at the 5% confidence level ($P \leq 0.05$). Spearman’s correlation and Principal Component Analysis (PCA) based on ANOVA were used to select the most significant variables from the
candidate variables of soil organic matter, microbial biomass, AMF spores, glomalin, humic acid, fulvic acid, humin, and soil porosity. This method allowed the variables to be evaluated for their ability to modify the aggregation of the soil. The analysis served to express the variables in terms of two components that explained the total variability associated with soil aggregation. Each component was accompanied by information about the correlation intensity and direction of the variable to the component. The results were illustrated in dispersion and loading-plot graphics created using Minitab 15 software.

RESULTS AND DISCUSSION

The soils from the experimental site showed a textural composition between sandy loam and silty loam, whereas the soil of the preserved site was predominantly loamy. Soil fertility, based on cation and organic matter availability in the experimental site, was low prior to transplantation in comparison to the preserved site (Table 1). The content of soil organic matter in the soil increased over time, regardless of the treatment, and had significantly low values in the disturbed site. Similarly, the soil CEC increased at 24 months after transplantation, reaching values equal to those found at the preserved site. This increase can be explained only by functional changes in SOM with the input of plant biomass as showed in literature.

Indeed, at 24 months after transplantation, the inoculated plots showed a higher plant coverage than the non-inoculated plots (Figure 4A), especially with herbaceous species and grasses. Therefore, inoculation with arbuscular mycorrhizal fungi favoured the establishment of vegetation at the experimental site and this effect occurred despite the flood. The survival of individuals depends on their flooding tolerance (Williams 2005) and as predicted the woody species occupation was not modified by inoculation procedures but the inoculated woody species associated with the herbaceous plants may have played an important role in soil aggregation and in the control of laminar soil erosion, as described in the literature (Rillig et al. 2003). The rehabilitation procedures increased the macroaggregates or larger aggregates production (Figure 4B) at the experimental site, which may be an indicator of erosion control (Bartheès & Roose 2002). Microaggregates are faster and more easily taken away by erosion processes than larger macroaggregates (Šimanský 2011). In the first

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>OM (%)</th>
<th>CEC (mmol/dm³)</th>
<th>SB (mmol/dm³)</th>
<th>V (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uninoculated</strong></td>
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<tr>
<td>6 months</td>
<td>7.70</td>
<td>6.83</td>
<td>15.16 a</td>
<td>2.34 a</td>
<td>7.33 b</td>
</tr>
<tr>
<td>24 months</td>
<td>7.33 b</td>
<td>6.83</td>
<td>15.16 a</td>
<td>2.34 a</td>
<td>7.33 b</td>
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<td><strong>Inoculated</strong></td>
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<tr>
<td>6 months</td>
<td>7.15</td>
<td>7.26</td>
<td>15.16 a</td>
<td>2.34 a</td>
<td>7.33 b</td>
</tr>
<tr>
<td>24 months</td>
<td>8.67 b</td>
<td>7.26</td>
<td>15.16 a</td>
<td>2.34 a</td>
<td>7.33 b</td>
</tr>
<tr>
<td><strong>Preserved site</strong></td>
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<tr>
<td>6 months</td>
<td>6.90</td>
<td>8.00 b</td>
<td>15.16 a</td>
<td>2.34 a</td>
<td>7.33 b</td>
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<tr>
<td>24 months</td>
<td>7.34</td>
<td>8.00 b</td>
<td>15.16 a</td>
<td>2.34 a</td>
<td>7.33 b</td>
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<tr>
<td><strong>Disturbed site</strong></td>
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<tr>
<td>6 months</td>
<td>7.35</td>
<td>10.00 b</td>
<td>15.16 a</td>
<td>2.34 a</td>
<td>7.33 b</td>
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<tr>
<td>24 months</td>
<td>112 b</td>
<td>10.00 b</td>
<td>15.16 a</td>
<td>2.34 a</td>
<td>7.33 b</td>
</tr>
</tbody>
</table>

OM – organic matter; SB – sum of bases; CEC – cation exchange capacity; V – base saturation; means with different letters are significantly different as determined by Tukey’s Multiple Range test at the 5% confidence level (P ≤ 0.05)
6 months after transplanting, microaggregates or small aggregates predominated at the experimental site. In spite of the flooding impact, a change in the aggregate profile occurred at 24 months after transplantation, with a significant improvement in the abundance of aggregates > 250 µm in inoculated plots. No aggregates were found at the disturbed site, and the largest aggregates (> 250 µm) were found to be more frequent at the preserved site (Figure 4B).

The total spore number was not significantly different between the inoculated treatments at 24 months after transplantation (Figure 5A) suggesting the flood effect on the AMF establishment. However, the spore number of both the Acaulosporaceae and Glomaceae AMF families increased in the inoculated plots (Figure 5A). Literature confirms that AMF propagules can tolerate the flooding effect (Harner et al. 2011). The significant increase of Acaulosporaceae and Glomaceae

![Graph]

Figure 4. An occupation index of plants (%) on the experimental area 24 months after transplantation (A) and soil aggregates distribution (%) in the experimental area at 6 (T1) and 24 (T2) months after planting compared with preserved site considering all classes > 250 µm and ≤ 250 µm (B); means with different letters are significantly different as determined by Tukey’s Multiple Range test at the 5% confidence level (P ≤ 0.05).

![Graph]

Figure 5. Soil arbuscular mycorrhiza fungi (AMF) community: total number of spores, Acaulosporaceae and Glomaceae families (A), microbial biomass (B) and soil glomalin content (C) from experimental soil (AMF inoculated and uninoculated plots) and reference sites 6 and 24 months after transplantation; means with different letters are significantly different as determined by Tukey’s Multiple Range test at the 5% confidence level (P ≤ 0.05).
spores speaks in favour of the flood tolerance of these families. AMF can favour not only plant growth, but also the input of litter and microbial decomposers (Scotti & Correa 2004). However, the microbial biomass did not differ between the inoculated and uninoculated plots (Figure 5B), as well as total AMF population. Although bacterial population is considered a transient agent of aggregation, roots and hyphae from fungal community, particularly arbuscular mycorrhizal hyphae, are considered as temporary binding agents that are able to persist for months and years, binding microaggregates to form macroaggregates (Six et al. 2000; Rillig et al. 2010). On the other hand, glomalin produced by AMF hyphae was significantly higher in inoculated than uninoculated plots (Figure 5C). This glycoprotein is considered a special agent of soil aggregate stability (Wright & Upadhyaya 1998) which is affected by soil management (Nichols & Millar 2013). Therefore, the increase in the population of some AMF families and soil glomalin content after 24 months at the experimental site could be related to an improvement in soil aggregation.

The rehabilitation procedure improved SOM content but there was no difference between the inoculated and uninoculated plots (Figure 6A). The soil organic matter content is not always related with soil aggregation and even a negative correlation was recorded between SOM and macroaggregates (Rokosch et al. 2009). However, the persistent binding agents as humic acids and glomalin are correlated to aggregate stability cementing the inter-particle bonds of microaggregates (Edwards & Bremner 1967; Wright & Upadhyaya 1998).

The recalcitrant or humified fraction is the most stable final product of lignin decomposition and is able to improve aggregate stability (Piccolo & Mbagwu 1994). Our results showed that cultivation promoted humic acid formation (Figure 6B) at 24 months after transplantation with a significant contribution of AMF inoculation. The fulvic acid content (Figure 6C) was also improved in both the inoculated and uninoculated plots compared to the disturbed site. The lack of treatment effect can be associated with the flooding effect. In general, humic substances (HS) from a river or wet sites under periodic flooding are comprised of molecules of smaller size, less condensed and more water soluble as fulvic acids (Spaccini & Piccolo 2009).

In fact, the improvement in soil aggregation indicates a considerable contribution of humic acid and glomalin especially in inoculated plots where an increase of plant biomass was also registered.

The treatment-independency of some studied variables as soil organic matter, total AMF spores,

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microbial biomass, and fulvic acid content could be attributed to the flooding effect characterized by the mixture of soluble elements among treatments and plots. Flooding can also alter the dynamics of all soil biological cycles and their products, therefore modifying not only the number but also the function of microbial species (Glazebrook & Robertson 1999). These results reinforce the hypothesis of tolerance and stability of soil aggregates facing flooding effect and the role of their forming agents as soil glomalin and humic acid. The mycelium of AMF fungus can maintain water-stable soil aggregates through increased soil water repellency of their hyphae (Rillig et al. 2010), what can explain the improvement of flood tolerance in the inoculated plot.

While at the preserved site a balanced distribution between microporosity and macroporosity (24 and 25%, respectively) was found, at the disturbed site the contribution of macroporosity to total porosity was twice the microporosity (15:36.3%, respectively). The same tendency was found at the experimental site 6 months after transplantation where the proportion of macroporosity was twice the microporosity (39:13%, respectively). The cultivation procedure favoured the desired distribution of macro- and microporosity at the experimental site 24 months after transplantation when the proportion between macroporosity and microporosity reached 27:21%, respectively. The increase in microporosity could be attributed to the aggregates formation. However, this improvement was independent of the inoculation treatment. The balance in soil porosity is essential for water drainage into soil under flood. Moreover, soil aggregates control porosity and therefore aeration and soil water drainage. There is a direct relationship between the quantity of macropores and micropores and soil water infiltration (Eynard et al. 2004). The improvement in microporosity at the experimental site under flooding effect suggests once again the stability of aggregates.

The results of PCA confirm that all of the studied variables (soil organic matter, AMF community, humic acids, glomalin, and microbial biomass) contributed to explaining 55% of the variation in soil aggregate formation, in addition to the vegetation effect. Therefore, the improvement in soil aggregation was a result of all of the studied variables under natural conditions. Including the second component (soil porosity), 72% of the soil aggregation variability was explained.

The scatter plot (Figure 7) was generated based on the variables that modified the aggregation such as component 1 (organic matter, humic acids, AMF fungi, glomalin, and microbial biomass) against the porosity variable (component 2). Based on the soil aggregates formed (Figure 7), several samples from the experimental site were grouped close to the samples from the preserved site. In contrast, other samples were placed between the preserved and disturbed sites and were influenced by both components 1 and 2. Another group formed by samples from the inoculated and uninoculated plots was clustered separately. The latter could be the result of the flooding impact because there was a strong influence of soil porosity (component 2).

These results showed that the characteristics of the recovering site are evolving toward those of the preserved site and that the rehabilitation process has attained an intermediate phase of restoration. This state of the process can be understood as a result of soil macroaggregate stabilization due to humic acid and glomalin action and all of them can be considered indicators of slope rehabilitation.

CONCLUSIONS

Arbuscular mycorrhizal fungi inoculation favoured the establishment of native species during slope rehabilitation and improved soil occupation as well as soil aggregates formation. Soil aggregates were considered an important indicator of slope rehabilitation.
when compared to the reference site which was able to withstand the environmental impact of flooding. The soil aggregation improvement can be attributed to the glomalin and humic acid contributions especially in inoculated plots and this process is indicative of the direction of the restoration evolution. Therefore, the AMF inoculation of woody and herbaceous species could be recommended in slope rehabilitation procedures.

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