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Terracing Reduces Arbuscular *Mycorrhizal fungi* Spore Loss through Surface Runoff

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HIGHLIGHTS

- The terracing reduces the peak flow of surface runoff.
- The terracing reduces the duration of the surface runoff period.
- The lower flow and duration of surface runoff decrease the loss of AMF spores.

Abstract: Surface runoff in agricultural areas promotes the transport of biological structures, such as arbuscular mycorrhizal fungi (AMF) spores, along with mineral particles, nutrients, and organic matter. Limited information exists regarding the relationships between the erosive process and the loss of AMF spores at the hillslope scale, as well as the effect of terracing on these relationships. The objective of this study was to quantify the loss of AMF spores in surface runoff water from agricultural soil managed under no-till and terraced conditions. The research was conducted in the Ribeirão Vermelho watershed, in a hillslope area in Cambé, Paraná, Brazil. Two experimental plots measuring 2.5 hectares each were installed, with one managed without terraces (NTP) and the other with level terraces (TP). At the lower end of the plots, a runoff flow measurement structure (H-channel) was installed to determine the flow rate and suspended spore count. The runoff duration, peak flow rate, and spore count were determined during the period from October 2019 to October 2022. The terrace reduced over 35% of suspended spore loss, and the hysteresis pattern showed a counterclockwise loop in NTP and a clockwise loop in TP for events with higher peak flow rates. The hysteresis pattern indicated rapid spore mobilization and transport in TP, suggesting that spores originated from more distant sources or required more energy for transportation in NTP. Overall, there is evidence that the terrace influences spore variability on the hillslope, reinforcing the need for terracing in agricultural hillslope areas.

Keywords: soil conservation; no-till; water erosion; mycorrhiza.

INTRODUCTION

Soil erosion in cultivated agricultural areas ranks among the primary causes of soil degradation, resulting in significant environmental and socio-economic losses [1]. This erosion occurs through the formation of surface runoff, leading to the detachment and transportation of soil particles. Consequently, the fertile topsoil layer is progressively lost, leading to soil impoverishment and a decline in agricultural productivity. Moreover, surface runoff carries away not only soil but also water, nutrients, mineral fertilizers, organic matter, and biological structures [2,3], including arbuscular mycorrhizal fungi (AMF) spores. These AMF spores serve as valuable indicators to assess soil biological quality [4] and erosive processes [5].

The loss of AMF spores through surface runoff can have detrimental effects on soil and plant health. Within plants, AMF play essential roles in enhancing water [6] and nutrient [7] uptake, increasing resistance against pests and pathogens [8], and improving resilience during drought [9] and low-temperature periods [10], among other benefits [4]. In the soil, AMF contribute to improved structure due to increased particle aggregation [11] and aid in heavy metal detoxification [12]. Consequently, the loss of AMF spores due to water erosion diminishes the potential soil inoculum, impacting mycorrhizal colonization, and subsequently affecting the benefits provided to both plants and soil.

Currently, in Brazil, more than 60% of the total area of annual crops is managed under the no-till system (NT), with an 84.9% increase in the area managed under this system from 2006 to 2017 [13]. However, NT areas frequently experience erosion, indicating that adopting this management system as the sole conservation practice is insufficient to ensure the sustainability of production systems, necessitating the combination of other soil and water conservation practices [13-15].

One mechanical practice of soil and water conservation is terracing, which has been used for water conservation, erosion reduction, and soil recovery, as observed by Londero and coauthors [16] in Southern Brazil. Recently, it has been noted that this practice improves other ecosystem services, such as carbon sequestration, soil fertility, and food security [17]. Notably, terracing also contributes to controlling the loss of AMF spores through erosion. However, research exploring the alterations caused by surface runoff in the soil's microbiological attributes, and the presence of AMF spores in the solution of surface runoff in agricultural hillslope areas, remains limited. Understanding the dynamics of the erosive process at the hillslope scale and its impact on AMF spores is crucial for effective water resource management, maintaining soil health, and optimizing production systems.

Through monitoring flow and sediment, hydrographs and sedimentograms can be determined, allowing the assessment of hysteresis between these variables at the hillslope or watershed scale [16,18]. The concentration of suspended sediment during the rising phase of the hydrograph may differ from that during the recession, reflecting the influence of soil conditions and sediment transport capacity. A similar phenomenon can be expected in the relationship between flow and AMF spores. This enables the evaluation of the temporal and spatial relationship between these hydrological and biological variables during the erosive process, thus discretizing the behavior of AMF spores in response to flow dynamics on a hillslope.

The hypothesis of this study is that surface runoff plays a significant role in the loss of arbuscular mycorrhizal fungi spores, and that conservation practices, such as the no-till system and terracing, contribute significantly to reducing the loss of this soil inoculum potential. The objective of this study is to evaluate the effect of terracing on AMF spore losses by monitoring surface runoff resulting from rain events during the period from 2019 to 2022.

MATERIAL AND METHODS

Study area

This study was conducted in the Ribeirão Vermelho watershed, located on a 220 m hillslope in the municipality of Cambé, Paraná, Brazil. The area lies in the second plateau of Paraná and has a Cfa-subtropical humid climate, with an average temperature of 20.4°C and an annual precipitation of 1,466 mm [19]. The experiment was established in 2018 on soil classified as typical Dystroferric Red Latosol at the hilltop and typical Dystroferric Red Nitosol at the foothill [20], with a high clay content (>600 g kg^{-1} of clay). The hillslope features undulating to gently undulating relief, with slopes ranging from 2% to 18% and a linear-convex profile.

Hillscope plot installation

The digital elevation model of the watershed encompassing the hillslope was used to select two experimental plots with similar topographical (slope, extension, and plan curvature) and hydrological features (accumulated flow and flow direction) as well as soil characteristics. Homogeneity was ensured both within and between the plots. After analyzing the topographical and hydrological attributes, the plots were delimited based on natural landscape boundaries, each covering an area of 2.5 ha. To isolate each plot and prevent external erosive processes, ridges were constructed along the lateral, upper, and lower limits.

Both plots have been managed under the no-till system for over 25 years, with soybean (*Glycine max* L.) cultivation in summer and second-season corn (*Zea mays* L.) in winter, and occasional scarification every three or four years. The plots were named terrace plot (TP) and non-terrace plot (NTP). In the NTP, pre-existing terraces were plowed down with disc harrows, while the TP had five broad-based terraces (upper and middle thirds) and one narrow-based terrace (lower third) sized using the curve number method [21]. Construction of the terraces was completed in August 2017, with the broad-based terrace established using a tractor-drawn 3-disc plow and a rear blade, and the narrow-based terrace constructed using a front-end loader-type tractor.

Precipitation, slope, soil class, land use, and soil management information were used to calculate the distances between terraces [22]. Precipitation was calculated using a 24-hour duration and a 10-year return period [23], and the infiltration rate was measured by the double-ring method [24] at 15 points within the plot, with a median value of 30 mm h^{-1}. For the terrace design, a contributing area and slopes of 5% (upper and middle thirds) and 12% (lower third) were considered. A surface runoff flow measurement structure, consisting of an H-channel, was installed at the lower end of each plot. Rain events were monitored from 2019 to 2022.

Determination of hydrological and biological variables

The variables obtained for each rain event that generated surface runoff included precipitation (ppt, mm), flow rate (Q, Ls^{-1}), runoff time (RT, minutes), the number of suspended AMF spores (nSpores, spores L^{-1}), and the total spore loss in each plot, according to the following equation:

$$Total\ spore\ number = k\sum(Q_i.nSpores_i)$$

where Q_i is the instantaneous water flow (L s^{-1}) and $nSpores_i$ is the instantaneous concentration of suspended spores (spores L^{-1}), and k is the unit conversion factor.

AMF spores were extracted through wet sieving, as described by [25], followed by sucrose flotation [26], and counted using a stereoscopic microscope.

Qualitative analysis of hysteresis

Hysteresis was assessed by determining time series that discretize the variation of flow values and the number of suspended spores over time [18]. The hysteresis curve characterization was obtained by graphing the number of spores as a function of flow data. The hysteresis loop, which can have clockwise, counterclockwise, or figure-eight patterns, characterizes the dynamics of spore entrainment as a function of flow in each plot. Hysteresis analysis was performed on the two largest and two smallest events to identify possible differences in this process between the plots.

Statistical analysis

Data normality and homogeneity of variances were assessed using Shapiro-Wilk and Levene tests, respectively, for the number of spores per event, total runoff time, and maximum event flow. As the data did not meet these assumptions, the medians were compared using the non-parametric Mann-Whitney test (p-value ≤ 0.05).

RESULTS

From October 2019 to October 2022, was monitored 103 rainfall events with a maximum precipitation of 73.0 mm and a minimum of 1.3 mm. Among these events, five generated surface runoff on May 28, 2019, October 23, 2021, and October 6, 18, and 20, 2022. The two events with the highest maximum flow rates occurred on October 23, 2021, with a precipitation of 49.4 mm and a maximum intensity of 188.9 mm h^{-1}, and on October 18, 2022, with a precipitation of 29.0 mm and a maximum intensity of 76.7 mm h^{-1} (Table 1). In these events, the peak flow rates in the non-terrace plot (NTP) were 89.5 L s^{-1} and 220.7 L s^{-1}, and in the terrace plot (TP) were 57.3 L s^{-1} and 34.1 L s^{-1}, respectively.

Table 1. Precipitation volume (ppt) and maximum intensity (Imax) of surface runoff-generating events in the period from 2019 to 2022.

Date	**Ppt (mm)**	**Imax (mm h^{-1})**
May 28, 2019	41.0	77.2
October 23, 2021	49.4	188.9
October 6, 2022	29.0	104.0
October 18, 2022	29.0	76.7
October 20, 2022	24.5	63.3

In the event on May 28, 2019, the precipitation was 41.0 mm with a maximum intensity of 77.2 mm h^{-1} (Table 1). However, the surface runoff flow in both plots was less than 25.0 L s^{-1}. In the events on October 6 and 20, 2022, the maximum surface runoff flow was below 10.0 L s^{-1} in both plots. The data for runoff time, maximum flow rate, and total number of spores lost in the TP and NTP are presented in Figure 1.

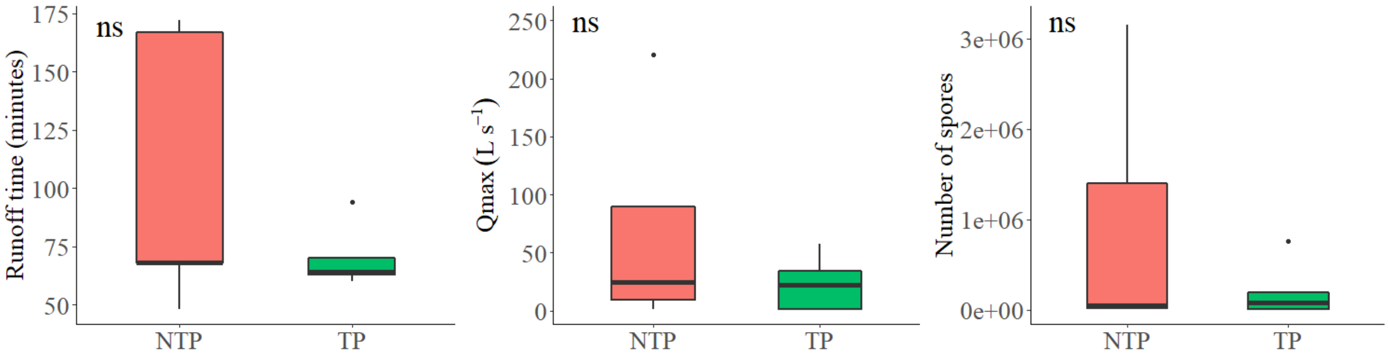

Figure 1. Runoff time, maximum flow rate (Qmax), and total number of arbuscular mycorrhizal fungi spores lost from the five events during the period between 2019 and 2022, in the terrace plot (TP) and non-terrace plot (NTP). "ns" indicates not significant.

There was no significant difference in runoff time (RT), maximum flow rate (Qmax), and spores number (nSpores) between TP and NTP. However, Figure 1 shows a wider range of RT, Qmax, and nSpores in NTP. In this plot, the highest surface runoff flow rate was 220 L s^{-1}, compared to TP, which had a maximum flow rate of 34 L s^{-1} on October 18, 2022. Besides the higher flow rate, NTP also exhibited a broader range of surface runoff duration data, with a duration of more than 100 minutes in two out of the five events (in the two largest events on October 23, 2021, and October 18, 2022, with 167 and 172 minutes, respectively), while the surface runoff duration in TP was at most 94 minutes, occurring in the event on October 18, 2022, and less than 70 minutes in the other four events.

There was no difference in the nSpores between the samples collected during each change in water level between the plots, even with the higher flow rate observed in NTP compared to TP. However, the longer duration of surface runoff in NTP resulted in a higher total spore loss, with medians of 7.49 × 10^{4} AMF spores lost in the surface runoff solution in NTP and 4.76 × 10^{4} AMF spores in TP. It is noteworthy that in NTP, the events on October 23, 2021, and October 18, 2022, resulted in a loss of more than 1 × 10^{6} spores, whereas in TP, the maximum number of spores lost per surface runoff was less than 8 × 10^{5} in these same two events of higher rainfall and flow intensity (Figure 1).

Graphs were constructed for the two largest and two smallest rainfall events that caused surface runoff and AMF spore loss (Figure 2).

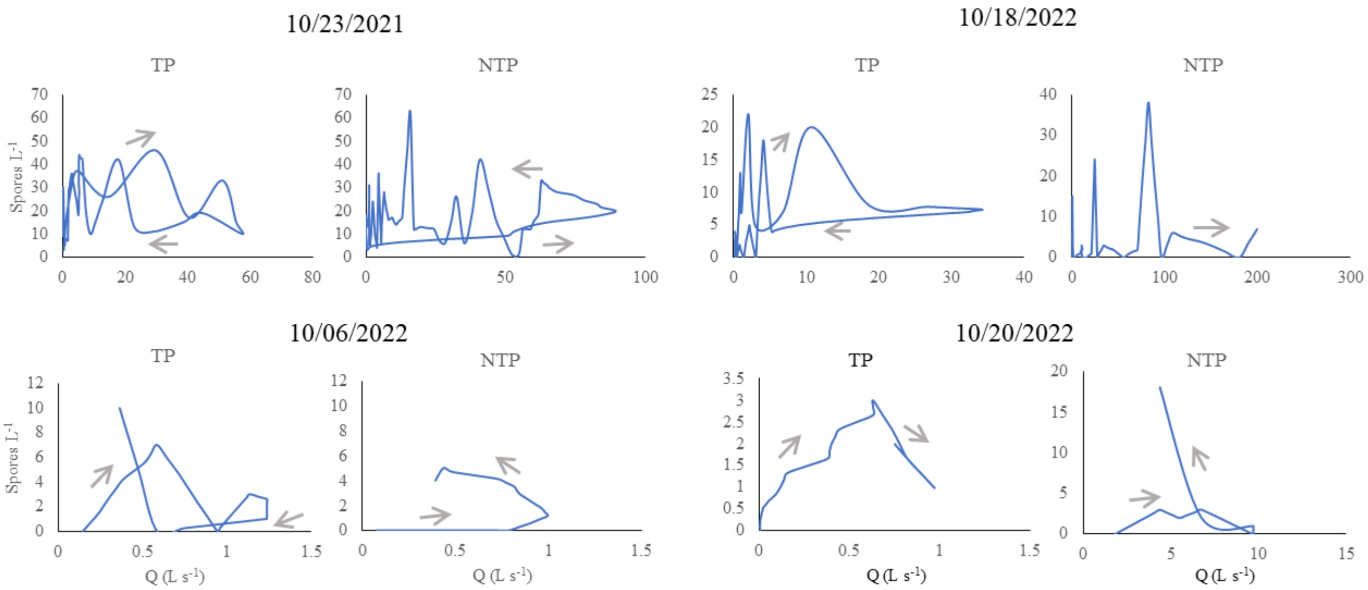

Figure 2. Hysteresis loops of events with the highest (October 18, 2022, and October 13, 2021) and lowest (October 06, 2022, and October 20, 2022) flow rates in plots with (TP) and without (NTP) terraces.

From the graphs, it can be observed that in TP, the hysteresis loop showed a clockwise pattern in both the events with the highest flow rates (October 18, 2022, and October 13, 2021) and the events with the lowest magnitudes (October 06, 2022, and October 20, 2022) (Figure 2). Conversely, in NTP, the loop consistently exhibited a counterclockwise pattern in these same events (Figure 2).

DISCUSSION

From the monitored events, it is evident that they can be classified as having high intensity and erosive characteristics. Alves and coauthors [27] propose that an event is erosive when the intensity within 30 minutes (I30) exceeds 13.3 mm h^{-1}. The I30 values of the monitored events were higher than 14 mm h^{-1}, with the lowest I30 occurring on October 20, 2022, at 14.2 mm h^{-1}, and the highest I30 on October 23, 2022, reaching 37.0 mm h^{-1}. The observed I30 values in these events substantiate the formation of surface runoff and the entrainment of AMF spores on the slope. Notably, all events generating surface runoff occurred in the month of October, except for the event on May 28, 2019.

Regarding the cultivation system on the slope, it does not involve practices such as straw production, crop rotation, or soil management. Coincidentally, October marks the period between winter crop harvest and soybean sowing for the summer crop. Consequently, throughout this month, the soil maintains low vegetative cover and straw content (< 7 Mg ha^{-1}) over the years. In a slope featuring Oxisols, Deuschle and coauthors [28] previously observed that a no-till system with less than 7 Mg ha^{-1}, similar to the one studied here, resulted in the highest soil and water losses, as well as peak flow rates, when compared to a no-till system that included crop rotation and straw production (>12 Mg ha^{-1}) for soil cover.

Comparatively, the peak flow rate in non-terraced plots (NTP) was 11 to 500% higher than in terraced plots (TP). Considering the similarity of soil classes on the slope and the agricultural management in the plots, it can be inferred that the presence of terraces reduced the amplitude of the maximum flow rate in TP (Figure 1). This reduction occurred because the terraces sectioned the slope, reducing the slope length, thereby attenuating the flow velocity and subsequently, the peak flow rate of surface runoff, effectively controlling the process of water erosion [29]. Numerous other studies demonstrate the effectiveness of terracing in controlling water erosion [30, 31, 32]. Additionally, peak flow rates exceeding 200 L s^{-1} in erosion studies on slope scales containing Oxisols under no-till systems without terracing were also observed by Londero and coauthors [16].

Consequently, the attenuation of the peak flow rate by terracing reduced the amplitude of the surface runoff duration in TP by over 200%, with the duration ranging from a minimum of 60 minutes to a maximum of 94 minutes in the five monitored events. Conversely, in NTP, these values ranged from 48 to 172 minutes.

Remarkably, the two largest events, occurring on October 23, 2021, and October 18, 2022, accounted for over 92% of all AMF spores lost in the five events that generated surface runoff. During these events, the terraces reduced the number of spores carried by erosion by over 300%, resulting in a reduction from 1.4 ×

10^6 AMF spores in NTP to 1.9 × 10^5 spores in TP on October 18, 2022, and a reduction from 3.1 × 10^6 spores in NTP to 7.6 × 10^5 spores in TP on October 23, 2021.

The qualitative analysis of the hysteresis graphs revealed that the pattern of entrainment and the controlling factors of the direction of the AMF spore hysteresis loop on the slope are influenced by the terraces. In TP, the hysteresis loop exhibited a clockwise pattern in both events with the highest flow rates (October 18, 2022, and October 13, 2021) and events with the lowest magnitudes (October 06, 2022, and October 20, 2022) (Figure 2). Conversely, in NTP, the loop consistently displayed a counterclockwise pattern in these same events (Figure 2).

The hysteresis loop in the clockwise direction indicates that the ascending branch of spore entrainment (spores-AB) during the increase in flow is greater than the descending branch (spores-DB), meaning that for a given flow rate (spores-AB > spores-DB). This type of hysteresis suggests that the spores are readily mobilized and transported even at low surface runoff flow rates during the event. This dynamic of AMF spore entrainment indicates that the peak spore entrainment occurs before the peak flow, and that the source of spores available for mobilization and transport in TP is near the H-channel and is exhausted during the event. This spore entrainment characteristic aligns with the effect of terraces on the slope, which section the slope with the presence of terraces, creating a steep (12% in the lower third of the plot) and short (approximately 32 m) drainage area, effectively bringing the source of spores close to the H-channel during surface runoff flow.

Conversely, in NTP, the counterclockwise hysteresis loop indicates that AMF spores-AB is lower than AMF spores-DB, meaning that for a given flow, AMF spore entrainment during the falling limb is higher than during the rising limb (AMF spores-AB < AMF spores-DB). This type of hysteresis suggests that the spores are not readily mobilized and transported, requiring higher surface runoff flow rates for spores to pass through the H-channel, as the spore concentration per liter of surface runoff increases after the flow peak (Figure 2). This spore entrainment dynamic suggests that the peak spore entrainment occurs after the flow peak, and that the source of spores available for mobilization and transport in NTP is farther from the H-channel.

It is important to highlight that the loss of AMF spores can reduce soil quality and impair the production system, considering that this fungus interacts with plant roots, increasing water and nutrient absorption [11] and directly contributing to soil structure formation, creating larger and more stable aggregates, and contributing to the formation of micro and macropores [11,33]. Thus, the greater loss of AMF spores in NTP may reduce the potential for inoculum, negatively affecting mycorrhizal colonization, and diminishing the benefits for the soil and plants provided by these fungi.

The result of spore loss in areas without terracing by surface runoff entrainment is consistent with the results obtained by Welemariam and coauthors [34]. These authors, comparing the traditional cultivation method without terracing to the use of terracing, found that areas with terracing not only had greater soil erosion control but also presented a higher number of AMF spores in the soil compared to areas without terracing, showing that terracing was effective in controlling spore loss through surface runoff entrainment. Additionally, Birhane and coauthors [35], in a study conducted in a pasture area in northern Ethiopia, found a higher spore density and greater root colonization in grasses in the terraced area compared to the pasture area without terracing. It was also observed that in the pasture area without terracing, in the lower portion of the slope, there was a higher amount of AMF spores than in the top and mid-slope, confirming that surface runoff indeed entrains fungal structures from the soil.

In addition to the presence or absence of terraces, the time period during which these structures remain in the soil is also important for AMF spores in the soil. Although newly constructed terraces prevent spore loss through surface runoff entrainment, the soil disturbance during their construction can provide unfavorable conditions for AMF development and negatively influence spore production. On the other hand, older terraces, in addition to acting as a physical barrier against surface runoff, preventing spore loss, have soil attributes restored from the disturbance during their construction, promoting an increase in organic matter and greater water retention capacity, factors that can positively impact spore production by AMF [35,36].

Habte [37] evaluated AMF spore loss in the soil through simulated rain and found that when the soil and fungal propagule loss was above 7.5 cm from the soil surface, significant spore losses and reduced mycorrhizal efficiency occurred. Losses of the soil layer less than 7.5 cm by surface runoff resulted in significant losses in spore abundance, but were not reflected in mycorrhization losses, suggesting that the AMF spores that remained in the soil were sufficient to colonize the plants in the area. Thus, the loss of the surface layer of soil by erosion directly influences the entire soil biodiversity, as well as the nutrients and organic material, not only affecting the AMF community.

The results found in this study are in line with several others in the literature and reinforce the need for terracing in sloping areas. Terracing is responsible for reducing surface runoff flow rates and, especially, reducing the duration of surface runoff, resulting in lower losses of soil structures due to water erosion, especially AMF spores.

CONCLUSION

The terrace system, when combined with no-till practices, acts as a physical barrier against water erosion, resulting in reduced flow rates and duration of surface runoff. The duration of surface runoff significantly influences the loss of arbuscular mycorrhizal fungi (AMF) spores caused by surface runoff. Terrace implementation shortens the duration of surface runoff, thereby reducing the loss of AMF spores. On the other hand, areas without terracing experience longer surface runoff periods, making them more susceptible to losses in the natural AMF inoculum in the soil, highlighting the necessity of constructing terraces on agricultural slopes managed under no-till systems.

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