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## Soybean supplementation increases the resilience of microbial and nematode communities in soil to extreme rainfall in an agroforestry system



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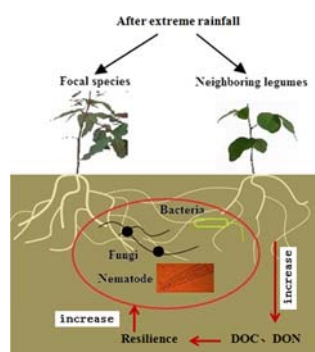
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### HIGHLIGHTS

- Intercropping with legumes increased the resilience of soil microbial community.
- Soil nematode community was more resilient in prickly ash and Soybean mixed culture.
- Plant composition was the driver to the resilience of soil biota communities.
- Soil dissolved organic carbon increased the resilience of biota communities.

### GRAPHICAL ABSTRACT



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### ABSTRACT

A current challenge for ecological research in agriculture is to identify ways in which to improve the resilience of the soil food web to extreme climate events, such as severe rainfall. Plant species composition influence soil biota communities differently, which might affect the recovery of soil food web after extreme rainfall. We compared the effects of rainfall stress up on the soil microbial food web in three planting systems: a monoculture of the focal species *Zanthoxylum bungeanum* and mixed cultures of *Z. bungeanum* and *Medicago sativa* or *Z. bungeanum* and *Glycine max*. We tested the effect of the presence of a legume on the recovery of trophic interactions between microorganisms and nematodes after extreme rainfall. Our results indicated that all chemical properties of the soil recovered to control levels (normal rainfall) in the three planting systems 45 days after exposure to extreme rain. However, on day 45, the bulk microbial community differed from controls in the monoculture treatment, but not in the two mixed planting treatments. The nematode community did not fully recover in the monoculture or *Z. bungeanum* and *M. sativa* treatments, while nematode populations in the combined *Z. bungeanum* and *G. max* treatment were indistinguishable from controls. *G. max* performed better than *M. sativa* in terms of increasing the resilience of microbial and nematode communities to extreme rainfall. Soil microbial biomass and nematode density

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were positively correlated with the available carbon and nitrogen content in soil, demonstrating a link between soil health and biological properties. This study demonstrated that certain leguminous plants can stabilize the soil food web via interactions with soil biota communities after extreme rainfall.

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## 1. Introduction

Agroforestry systems can promote agricultural production by minimizing nutrient loss, maximizing internal cycling of nutrients, improving the nitrogen (N) and carbon (C) content of the soil for C sequestration, enhancing soil biodiversity, and reducing the threat of extreme weather to target plants (Tsonkova et al., 2012).

A tree-based agroforestry system has been promoted as an ecologically-sustainable system that allows the use of conventional agricultural practices (Bainard et al., 2013). This system involves planting crops between widely-spaced rows of trees. The incorporation of trees as just one agroforestry property, can increase crop yields by reducing evaporation losses and by protecting of crops from excessive heat (Coulibaly et al., 2017). Moreover, crop yields are more stable under tree cover systems due to the ability of tree to modify microclimates by mitigating the effects of climate change, particularly extreme weather fluctuations within the cropping alley (Schoeneberger et al., 2012). However, the existence of trees in an agricultural system can also result in strong competition between trees and crops for soil nutrients and light (Friday and Fownes, 2002). These could, in turn, alter the resilience of soil microorganisms including microbial and nematode communities, to extreme weather fluctuation in a tree-based agroforestry system (Bainard et al., 2013). However, the consensus within the literature is that the positive aspects of tree cover to increase the general environment for crop growth becomes less suitable, especially when climate extremes increase (Lin et al., 2008). More studies are required to evaluate the specific impact of crops on the soil environment under extreme weather conditions. This will help to formulate more appropriate recommendations for tree cover management.

In tree-based agroforestry systems, the repeated decline of soil nutrients over time is inevitable, hence the introduction of leguminous plants, as an alternative to replenish the soil fertility, has become common-place, largely due to their ability to fix nitrogen (Gmezmuoz, 2014; Coulibaly et al., 2017). The presence of nitrogen-fixing leguminous plants has been reported to increase soil carbon and nitrogen content by increasing net primary productivity and the rapid turnover of high quality litter and root exudates (Zhao et al., 2014). Specifically, legumes can intensify the complexity of the soil environment (Gao et al., 2017). However, whether the intensified complexity of a soil environment can promote the resilience of microbial and nematode communities in soil to extreme climate stress is poorly understood. Different leguminous species may adopt differential strategies which may be more or less suited to mitigating the effects of extreme climate stress, such as heavy rainfall. For example, stressed plants tend to invest in defense compounds (e.g., phenols in leaves) and in storage compounds (e.g., root carbohydrates) to increase their resilience to short-term disturbance (Chen et al., 2013). The potential effect of plant compensation to water-saturated soil stress on soil communities may similarly build resilience, a phenomenon that necessitates further study.

Resilience has been well described in the literature as one of the components of ecological stability. Pimm (1984) defined resilience as how fast parameters return towards their equilibrium following a perturbation. Orwin and Wardle (2005) further presented a new resilience index by calculating the absolute difference that exists between disturbed soil and control relative to the initial absolute effect of disturbance. Griffiths and Philippot (2013) further suggested that engineering resilience is where the behaviour of a system is treated like an engineering material that will recover towards its

predisturbance state or a new stable state; this engineering resilience approach now predominates in studies of soil biology. More recently, Wagner et al. (2015) used the concept of proportional resilience to describe the ability of a system to reach its original condition prior to disturbance. In the present study, we defined resilience as when the individual species abundance, species composition, and total density or biomass returns to control levels. The use of control and disturbed samples at the same time could take into account any changes occurring in the control and disturbed soils over time in the field (Griffiths and Philippot, 2013).

The adaptations of plants to extreme climate stress could significantly change the allocation of carbon to defense and storage and can also alter litter quality and quantity (Mariotte et al., 2016). These changes have the potential to affect the resilience of the soil community (Griffiths and Philippot, 2013). Although microbial resilience to drought has been reported to be related to the biomass of plant shoots and roots in a legume monoculture (Orwin and Wardle, 2005), the benefit of plant cover upon the resilience of a soil community to extreme rainfall, which has been predicted to increase under climate change, remains ambiguous. In this study, we tested the resilience of a microbial and nematode community in soil to an extreme rainfall event in three different agroforestry systems with legumes. Moreover, little is known about the effects of crops within an agroforestry system upon the stability of microbial and nematode communities in soil, since previous studies mostly focused on how different species of trees in agroforestry management can stabilize primary productivity.

Soil microbial and nematodes occupy central positions in the soil biota community and are considered as representing sensitive indicators for changes in soil health (Tom and Howard, 1999; Wagner et al., 2015). Moreover, these organisms play roles in carbon and nitrogen cycling that are vital to agricultural productivity. Soil nematodes span several trophic levels and play an important role in nutrient flow by grazing microbes, decomposing organic material and preying upon other animals in the soil (Bardgett and van der Putten, 2014). Similarly, the composition of the soil microbial community plays a key role in the decomposition of plant materials, nutrient cycling, soil carbon storage, conservation of soil structure and primary productivity (Fang et al., 2016). It has been suggested that microbial resilience is positively related to the dissolved organic carbon content of soil (de Vries et al., 2012) and that supplements of organic matter increase nutrient acquisition and the resilience of microbes to drying-rewetting stress (Sun et al., 2017). The resilience of bacterial-feeding nematodes has also been suggested to be positively correlated with both the dissolved organic carbon content of soil (de Vries et al., 2012) and plant species (Viketoft et al., 2009; Cesarz et al., 2013), with the latter appearing to exert the strongest influence of the two. We sought to test whether the resilience of microbial and nematode communities in soil are more dependent on plant identity or soil properties, such as dissolved organic carbon and nitrogen.

This field study examined the resilience of microbial and nematode communities in soil to severe soil wetting in monoculture plantations of prickly ash and plantations mixed with leguminous species (alfalfa and soybean). We hypothesized that the presence of legumes would have a positive effect on the resilience of microbial and nematode communities in soil and that the effect would depend on the species of legume. Our results should shed light on our potential to strengthen the resilience of agricultural ecosystems using ecological principles and provide evidence for the application of agroforestry for leguminous crops.

## 2. Materials and methods

### 2.1. Study site

The experiment was conducted in Maoxian, Sichuan Province, southwestern China (103°53'E, 31°41'N), at an altitude of 1686 m. The mean annual temperature, precipitation and evaporation were 8.9 °C, 920 mm and 796 mm, respectively, according to meteorological monitoring data acquired from the Maoxian Ecological Station of Chinese Academy of Science. Most precipitation occurs during the months of August at this particular site and an increased frequency of extreme precipitation events has been reported in this area (Zhai et al., 2005). The soil is classified as Udic Luvisols (IUSS, 2007).

### 2.2. Experimental design

Agricultural soil containing organic fertilizer, normally used for cultivating vegetables, was applied at the start of the experiment. A randomized complete block design was used to establish the experiment in April 2013 (Sun et al., 2016). Three planting systems were implemented: (i) a monoculture of *Zanthoxylum bungeanum* (Z); (ii) a mixed culture of *Z. bungeanum* and *Medicago sativa* L. (Z-M) and (iii) *Z. bungeanum* and *Glycine max* (L.) Merr. (Z-G). A batch of uniform, two-year-old seedlings of *Z. bungeanum* were selected from a local nursery according to plant stem diameter at the soil surface and height. *Z. bungeanum* is a non-leguminous tree plant, while *M. sativa* and *G. max* are leguminous, herbaceous plant species. One *Z. bungeanum* was grown in the centre of each plot while species of *G. max* and *M. sativa* were planted at the same density (0.27 m<sup>2</sup>/individual) in all plots. Each plot area was 2.6 m × 2.6 m in size, and the distance between each plot was 1 m. Each treatment was replicated six times, making a total of 18 plots. No additional fertilization was applied after the experiment was commenced and weeding was performed by hand each week. Herbicide was not used to avoid unwanted effects on soil organisms. In August 2015, we exposed our plots, at random to precipitation treatment in which triplicate plots per each system received either normal rainfall (control) or extreme rainfall. Control and extreme rainfall plots were kept under rainout shelters during the experimental period (from 1st–30th August, August 2015) to control moisture. Tap

water was used to mimic extreme precipitation events, and a watering pot (the intensity of the watering was 3.53 L/min) was used to compensate for rain (Sun et al., 2016). Rain regimes were designated according to Ng et al. (2015), mirroring the mean rainfall of 3.0 mm/day in the local area during the month of August and an abnormally high rainfall for August (9.5 mm/day) (mean rainfall and extreme rainfall data calculated between 1983 and 2013 from the Maoxian Ecological Station of Chinese Academy of Science). During the month-long experimental period, all the plots were watered in the morning (7–9 am) and evening (6–8 pm). The shelters were constructed using steel frames and were covered with transparent plastic to protect plants from precipitation (Borken et al., 2006). To minimize greenhouse effects, the rainout shelters were situated 1.8 m aboveground. Control plots were watered until October 15th, but soil moisture in the extreme rainfall plots took 9 days longer to recover to the control level and were watered an equal amount as the control plots. Thick PVC panels were inserted to a depth of 0.5 m into the soil around each plot to prevent lateral water movement between plots and to prevent interactions with roots from neighboring plots.

### 2.3. Field sampling

Soil sampling was conducted on August 31st, September 15th, September 30th and October 15th 2015. A total of five soil cores (5 cm diameter), to a depth of 10 cm were collected using the five-spot method and amalgamated to form one sample per plot (Sun et al., 2016).

### 2.4. Soil properties

Soil water content (SWC) was estimated gravimetrically by oven drying (105 °C for 24 h) 20 g of a field soil sample. Soil ammonium (NH<sub>4</sub><sup>+</sup>-N) and nitrate (NO<sub>3</sub><sup>-</sup>-N) were extracted with 2 M KCl solution and measured by a flow injection autoanalyzer (AA3, Bran + Luebbe, Germany). Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were extracted from the soil with 2 M KCl solution and estimated using a TOC/TN analyzer (Multi N/C@2100(S), Analytik Jena AG, Germany).

**Table 1**  
Soil chemical properties at each sampling time. Statistically significant differences between the control and rainfall plots are shown in bold type ( $P < .05$ ). Different uppercase letters indicate significant differences among control (normal rainfall) treatments; different lowercase letters indicate significant differences among recovery treatments from extreme rainfall.

Recovery days	Planting system	Soil properties		Soil water content			
		Treatment	(%)	NH <sub>4</sub> <sup>+</sup> -N (mg/kg)	NO <sub>3</sub> <sup>-</sup> -N (mg/kg)	DOC (mg/kg)	DON (mg/kg)
0 day	Z	Control	<b>18.63 ± 0.80</b>	4.16 ± 0.41	<b>17.81 ± 0.45 A</b>	<b>212.88 ± 1.06 A</b>	55.25 ± 0.49 A
		Rainfall	<b>30.26 ± 0.15</b>	5.97 ± 0.66a	<b>9.62 ± 0.59</b>	<b>189.80 ± 4.43 a</b>	52.82 ± 0.80 a
	Z-M	Control	<b>19.42 ± 0.67</b>	6.07 ± 0.86	<b>24.94 ± 0.71 B</b>	237.94 ± 7.07 B	64.89 ± 2.25 B
		Rainfall	<b>31.82 ± 0.61</b>	9.18 ± 0.99b	<b>10.52 ± 1.01</b>	223.27 ± 5.53 b	62.04 ± 2.85 b
	Z-G	Control	<b>19.14 ± 0.54</b>	<b>4.31 ± 0.49</b>	<b>25.19 ± 1.12 B</b>	<b>229.37 ± 1.98 B</b>	62.83 ± 0.89 B
		Rainfall	<b>30.46 ± 0.69</b>	<b>9.04 ± 0.94b</b>	<b>11.30 ± 0.79</b>	<b>216.40 ± 4.11 b</b>	61.43 ± 2.91 b
15 days	Z	Control	<b>18.10 ± 0.53</b>	3.06 ± 0.15 A	<b>21.22 ± 0.70 A</b>	195.66 ± 9.98	49.88 ± 0.43 A
		Rainfall	<b>19.82 ± 0.29</b>	3.62 ± 0.25 a	<b>14.37 ± 0.66</b>	163.05 ± 13.26 a	48.76 ± 1.13 a
	Z-M	Control	19.74 ± 1.21	4.16 ± 0.28 B	<b>26.57 ± 2.40 AB</b>	263.98 ± 42.91	61.58 ± 4.50 B
		Rainfall	19.54 ± 1.61	5.17 ± 0.39 b	<b>15.09 ± 1.25</b>	201.83 ± 4.11 b	58.36 ± 0.71 b
	Z-G	Control	18.93 ± 0.94	<b>3.43 ± 0.19 AB</b>	<b>28.56 ± 2.01 B</b>	196.09 ± 13.38	55.99 ± 1.18 AB
		Rainfall	20.93 ± 1.45	<b>5.76 ± 0.41 b</b>	<b>16.77 ± 1.31</b>	182.48 ± 7.37 ab	51.90 ± 2.97 ab
30 days	Z	Control	18.03 ± 0.57	3.65 ± 0.18 A	18.72 ± 1.15 A	174.54 ± 15.17	51.79 ± 1.04
		Rainfall	17.82 ± 0.46	3.05 ± 0.23	16.18 ± 1.75 a	145.54 ± 8.87 a	49.10 ± 0.86
	Z-M	Control	19.34 ± 0.96	<b>4.65 ± 0.22 B</b>	27.97 ± 1.62 B	188.71 ± 11.75	58.00 ± 3.69
		Rainfall	19.14 ± 0.62	<b>3.49 ± 0.22</b>	25.87 ± 0.51 b	172.84 ± 5.68 b	56.15 ± 0.99
	Z-G	Control	19.24 ± 0.76	3.99 ± 0.22 AB	<b>25.76 ± 1.19 B</b>	173.52 ± 4.84	52.48 ± 4.58
		Rainfall	18.33 ± 0.98	3.65 ± 0.33	<b>18.35 ± 1.62 a</b>	173.63 ± 6.40 b	48.86 ± 3.35
45 days	Z	Control	17.91 ± 0.36	2.31 ± 0.62	18.57 ± 2.24	152.36 ± 2.17 A	48.04 ± 2.49
		Rainfall	17.12 ± 0.88	2.11 ± 0.49	16.8 ± 0.68 a	140.07 ± 8.80 a	44.03 ± 2.72
	Z-M	Control	19.45 ± 0.80	1.98 ± 0.66	23.25 ± 1.27	179.35 ± 5.66 B	52.89 ± 3.81
		Rainfall	19.06 ± 0.33	2.04 ± 0.49	22.62 ± 0.84 b	161.08 ± 6.53 ab	51.5 ± 1.53
	Z-G	Control	19.52 ± 1.19	2.27 ± 0.64	21.98 ± 2.54	165.24 ± 7.26 AB	53.2 ± 1.75
		Rainfall	18.47 ± 1.55	3.32 ± 0.29	20.01 ± 1.48 ab	171.84 ± 8.16 b	49.56 ± 3.13

2.5. Phospholipid fatty acid (PLFA)

Soil microbial biomass and community structure was assessed using PLFA profiles (Frostegård et al., 1996). Eight grams of dry-weight-equivalent fresh soil was used for the extraction of lipids in a 23-mL extraction mixture containing chloroform:methanol:phosphate buffer (1:2:0.8 v/v/v). The concentration of each PLFA was calculated based on a c19:0 internal standard and the abundance of the individual fatty acids was reported as nmol lipid per gram of dry soil. Total bacterial biomass was estimated based on the sum of i15:0, a15:0, 15:0, i16:0, 16:1ω7, i17:0, a17:0, 17:0, cy17:0, 18:1ω7c and cy19:0. Total fungal biomass was estimated based on the total of 18:2ω6, 9c PLFA (Frostegård et al., 1996).

2.6. Nematode community

The modified cotton-wool filter method was used to determine the number of nematodes per 50 g soil (Townshend, 1963). The total number of nematodes was counted and 100 specimens were randomly subsampled and identified using an inverted compound microscope. Nematode populations were expressed as the number of nematodes per 100 g of dry soil. Nematodes were categorized into four groups

according to their feeding habits (Yeates et al., 1993): (1) bacterivores; (2) fungivores; (3) omnivores-predators and (4) herbivores.

2.7. Statistical analyses

Statistical analysis was performed using SPSS v. 17.0 (SPSS Inc., Chicago, IL). Differences in soil properties, microbial biomass and nematode density between control and extreme rainfall treatments were tested by independent-sample *t*-tests. Principal component analysis (PCA) was performed to detect changes in the microbial and nematode community using all PLFA markers and nematode genera. The following variables were used as environmental variables in the PCA: NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup> N, DOC, DON and soil water content (SWC). The principal response curves (PRC) method (Lepš and Šmilauer, 2003) was used to determine the recovery trends of soil nematode populations (segregated by functional guilds) using CANOCO 4.5 software (Ithaca, NY, USA). PRC was based on redundancy analysis. Control treatments were assigned as the baseline, and recovery effect was represented by the deviation of each fluctuating line from zero. Pearson correlations were used to assess relationships between microbial biomass, nematode density and soil characteristics.

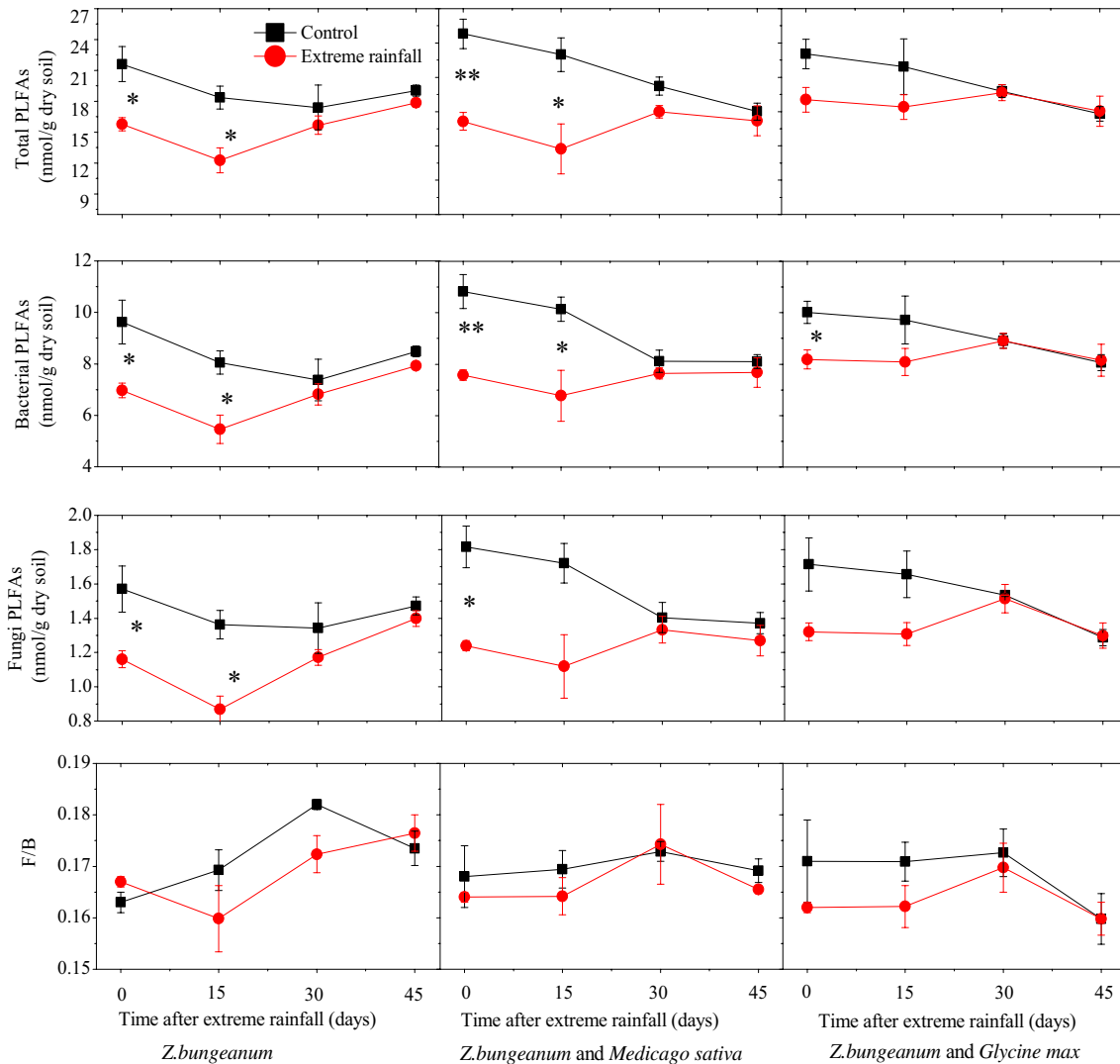


Fig. 1. Effects of the composition of plant species on the microbial community after 45 days recovery from extreme rainfall. Time zero is the time when extreme rainfall terminated. \**P* < .05, \*\**P* < .01 according to *t*-tests. Standard errors of the mean are shown as line bars. *n* = 3.

### 3. Results

#### 3.1. Soil properties

Extreme rainfall caused a significant reduction in soil  $\text{NO}_3^-$ -N content in all of the planting systems (Table 1). After 15 days of recovery,  $\text{NO}_3^-$ -N content was significantly lower in the extreme rainfall treatments ( $P < .05$ ). After 30 days, soil  $\text{NO}_3^-$ -N content was still significantly greater in controls in the mixed planting of *Z. bungeanum* and *G. max*. Forty-five days after the precipitation event, all soil properties were comparable between control and treated soils.

#### 3.2. Microbial community

Extreme rainfall caused a significant reduction in total microbial, bacterial and fungal biomass in monoculture and in the *Z. bungeanum* and *M. sativa* mixed culture ( $P < .05$ ; Fig. 1). Microbial biomass recovered to control levels after 30 days. Extreme rainfall significantly reduced bacterial biomass in the mixed culture of *Z. bungeanum* and *G. max* ( $P < .05$ ), but reached parity with the controls by day 15 (Fig. 1).

There was a strong difference in microbial community composition when compared between control and extreme rainfall treatments, as indicated by a clear separation along the second axis of the PCA (Fig. 2). By the end of the experiment, the PLFA marker 16:1 $\omega$ 7c had not recovered to the same level as controls in monoculture ( $P = .011$ ). Microbial community composition returned to control levels in both mixed culture treatments by the end of the study. Soil water content,  $\text{NO}_3^-$ -N, DOC and DON strongly influenced the composition of the microbial community (Fig. 2).

#### 3.3. Nematode community

Extreme rainfall caused a significant reduction in the number of fungivores in *Z. bungeanum* monoculture ( $P < .05$ ; Fig. 3), although populations recovered by day 45. Extreme rainfall also caused significant

reductions in bacterivore, fungivore and omnivore-predator density in both mixed culture treatments ( $P < .05$ ). In *Z. bungeanum* and *M. sativa* plots, only fungivore density returned to control levels after 45 days of recovery; the density of bacterivores and omnivore-predators did not fully recover. In *Z. bungeanum* and *G. max* plots, the density of bacterivores, fungivores and omnivore-predators all returned to control levels (Fig. 3).

PRC demonstrated the effective recovery of nematode populations and functional guilds by day 45 (Fig. 4). Although the trophic nematode composition in *Z. bungeanum* monoculture did not significantly differ between control and treatments after 45 days, certain functional guilds did not completely recover. The weight of *He*<sub>2</sub> gradually decreased ( $P = .073$ ), while that of *Ba*<sub>4</sub> increased gradually ( $P = .012$ ) after 45 days (Fig. 4a). The structure of the nematode functional guilds did not return to control levels in *Z. bungeanum* and *M. sativa* mixed culture (Fig. 4b). The weight of *He*<sub>2</sub> increased gradually ( $P = .055$ ) while that of *He*<sub>3</sub> gradually decreased ( $P = .057$ ) after 45 days. In contrast, the composition of nematode functional guilds matched the controls in *Z. bungeanum* and *G. max* plots after 45 days of recovery (Fig. 4c).

Rainfall treatment had a mixed effect on nematode population density. The density of nematodes in monoculture plots belonging to the genera *Tylenchus*, *Thonus* and *Aporcelaimellus* were higher than in controls ( $P \leq .05$  except for *Tylenchus* at  $P \leq .1$ ), while the density of *Psilenchus*, *Longidorus* and *Mesodorylaimus* was less than controls ( $P \leq .05$  except for *Longidorus* at  $P \leq .1$ ; Table A.1). In *Z. bungeanum* and *M. sativa* mixed culture, the densities of *Lelenchus*, *Pratylenchus*, *Odontolaimus*, *Anaplectus*, *Bastiania*, *Aphelenchus*, *Achromadora* and *Mesodorylaimus* were less than in the control plots ( $P \leq .05$  except for *Achromadora* at  $P \leq .01$ ) (Table A.2). The density of all nematode genera returned to control levels in the mixed *Z. bungeanum* and *G. max* plots (Table A.3).

Extreme rainfall also led to significant changes in the composition of the nematode community which were strongly influenced by soil water,  $\text{NO}_3^-$ -N, DOC and DON (Fig. 5). Only the nematode communities in mixed *Z. bungeanum* and *G. max* plots resembled the

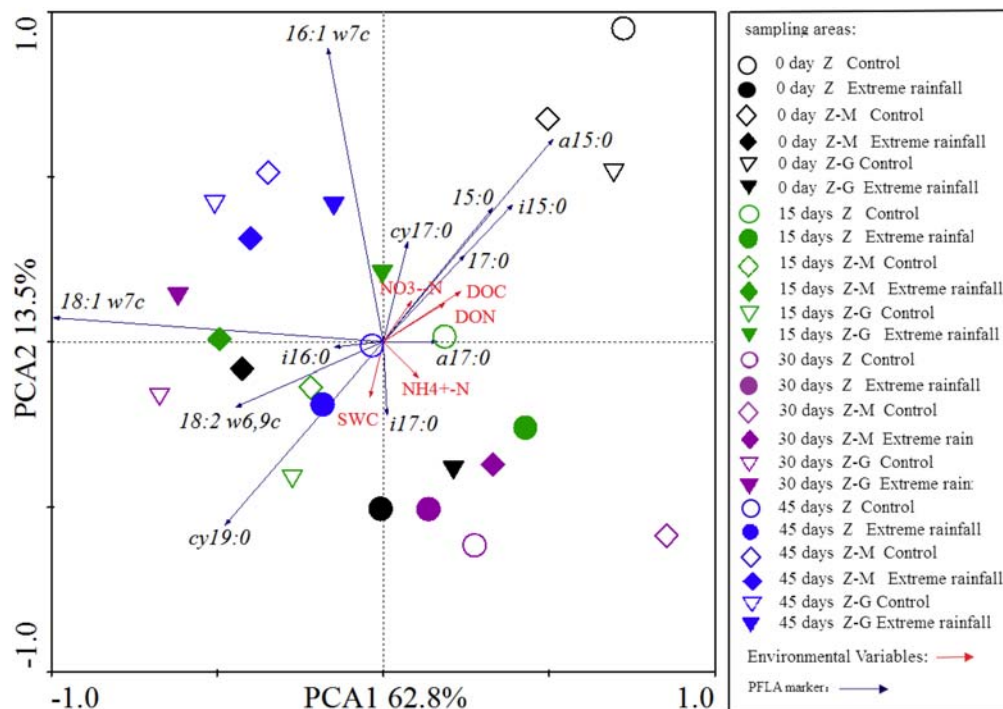


Fig. 2. Principal component analysis of the microbial community. Environmental variables are just projected in the graph: soil water content (SWC), dissolved organic carbon (DOC), dissolved organic nitrogen (DON).

composition of the controls. In monoculture, the densities of *Tylenchus*, *Acrobeloides* and *Thonus* nematode genera were higher than controls, while the relative abundance of *Psilenchus* was lower ( $P \leq .05$ ). In *Z. bungeanum* and *M. sativa* plots, the relative abundance of *Filenchus*, *Boleodorus* and *Tripyla* was higher than controls, while that of *Odontolaimus*, *Anaplectus* and *Achromadora* was lower ( $P \leq .05$ ).

3.4. Correlations between soil biota and soil properties

Total microbial, bacterial and fungal biomass along with total nematode and omnivore-predator densities were positively correlated with soil DOC, DON and  $\text{NO}_3^- \text{-N}$  (Table 2). Fungal biomass was negatively correlated with soil water content (Table 2). Bacterivores and omnivore-predator density was positively correlated with total microbial, bacterial and fungal biomass (Table 3).

4. Discussion

4.1. Resilience of soil microbial communities to extreme rainfall

Microorganisms were strongly affected by extreme rainfall, however, most measures, including microbial biomass, returned to control levels within 30 days. Pearson correlation analysis showed that soil DOC, DON and  $\text{NO}_3^- \text{-N}$  had substantial positive effects on microbial biomass, suggesting that contributions of DOC and DON from the plants were important drivers for the resilience of soil microbial productivity.

Extreme rainfall led to significant reductions in the bacterial biomass of all planting systems. Bacterial populations exhibited the strongest rate of recovery, probably owing to their fast rate of growth. Fungal biomass was also significantly reduced by extreme rainfall in monoculture and mixed plots of *Z. bungeanum* and *M. sativa* mixed culture, but returned to control levels by day 30. This was attributed to the fact

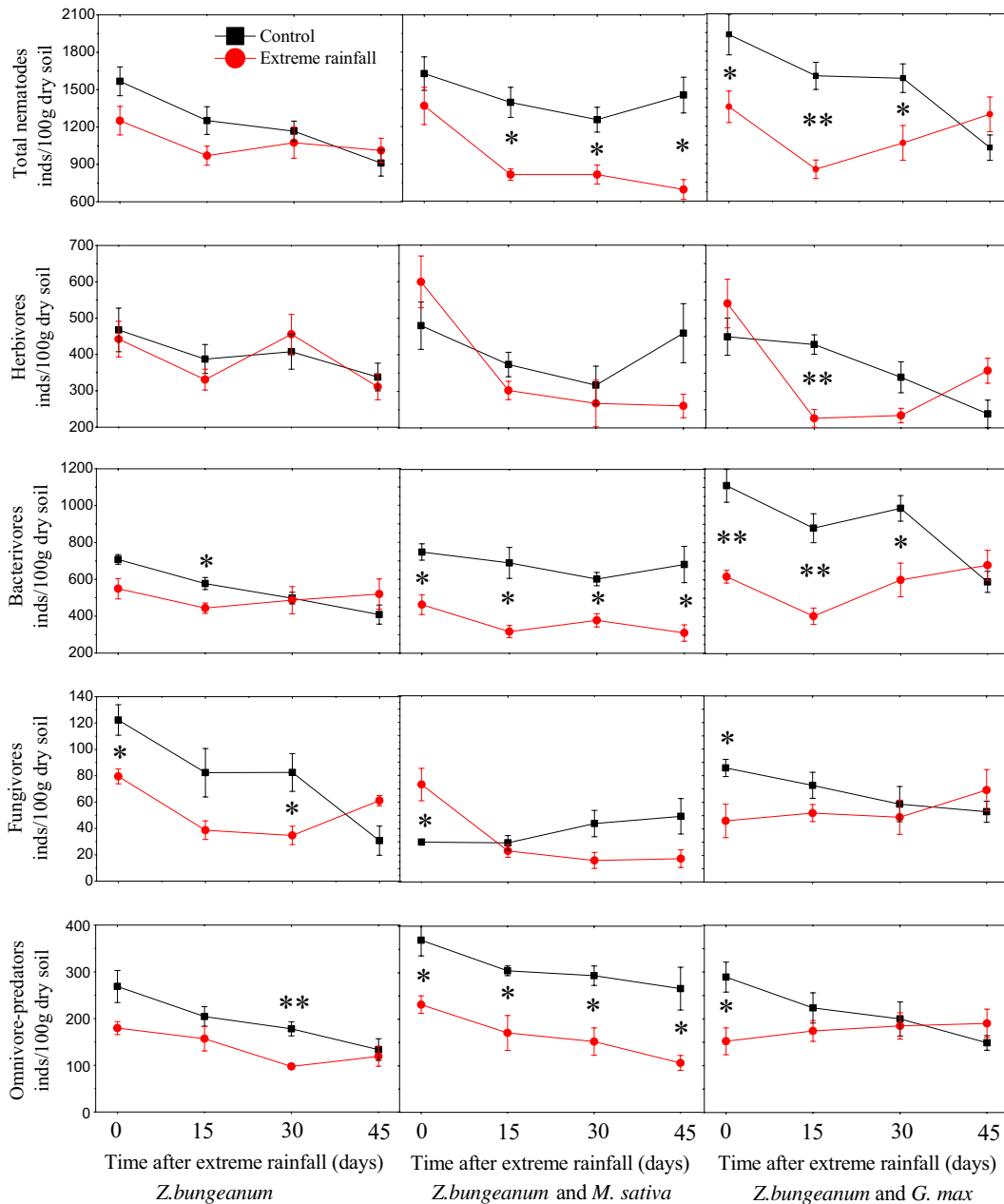
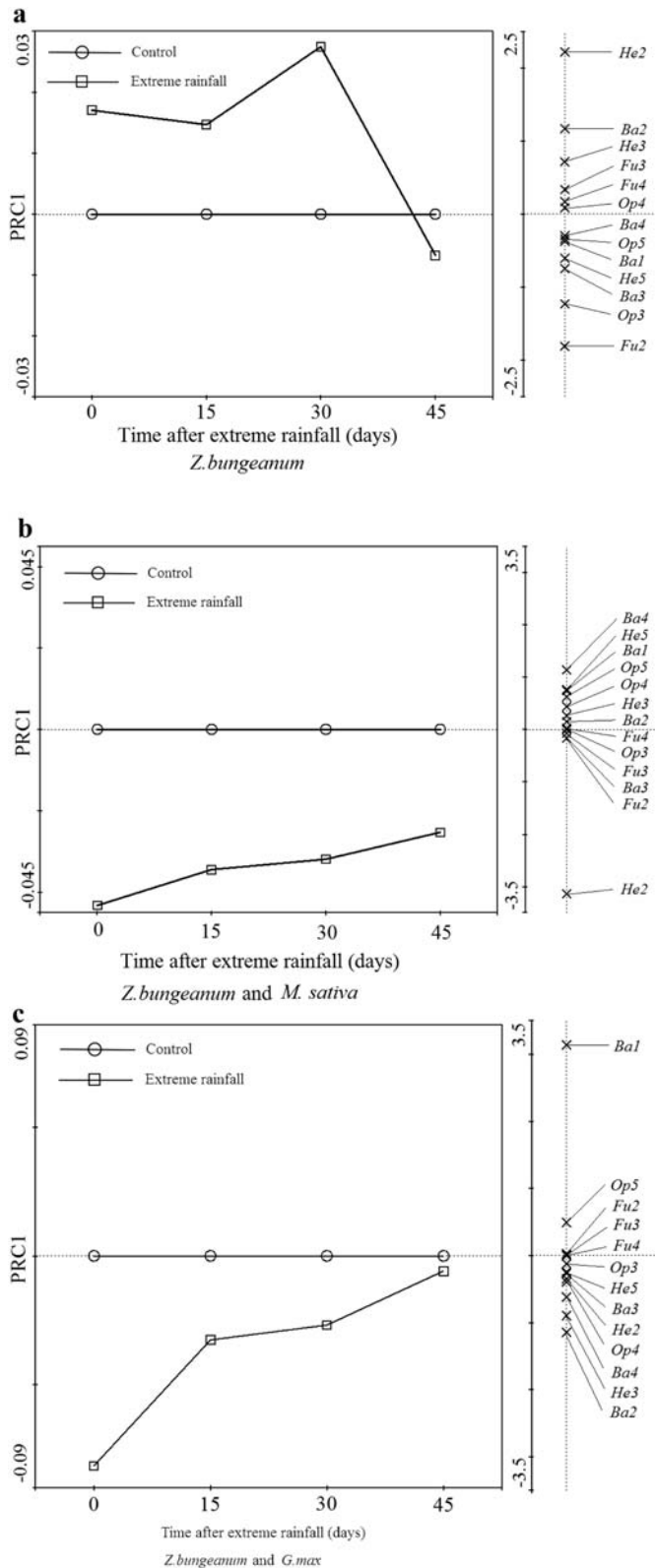


Fig. 3. Effects of plant species composition on the nematode density after 45 days recovery from the extreme rainfall. \* $P < .05$ , \*\* $P < .01$  according to  $t$ -tests. Standard errors were shown as line bars,  $n = 3$ .

that bacteria and fungi had proportionally similar turnover rates, with mean turnover times of  $11.0 \pm 1.0$  d and  $9.7 \pm 1.1$  d, respectively (Blazewicz et al., 2014). Thus, soil microbial biomass may be resilient



**Fig. 4.** Principal response curves with density weighting for each soil nematode functional guild in different treatment plots. The horizontal axis represents the control treatment. Soil nematode functional guilds are designated as: Ba, bacterivores; Fu, fungivores; OP, omnivore-predators; He, herbivores.

to extreme rainfall (Cruz-Martinez et al., 2009), and could return to control levels within 30 days once the soil had dried.

Extreme rainfall also caused shifts in the composition of the soil microbial community, especially the PLFA marker (cy19:0) known to correspond to anaerobic bacteria (Bossio and Scow, 1998). Although soil chemical properties returned rapidly to control levels in monoculture, the microbial community was delayed because of the time taken for a population to develop. All PLFA markers in mixed plots bounced back to control levels after 45 days, while the PLFA marker 16:1 $\omega$ 7c in monoculture did not recover to control levels (Fig. 2). This was consistent with our hypothesis that the presence of legumes will increase the resilience of microbial communities in soil. This effect may have resulted from the increased content of easily degradable organic materials, and the availability of root exudates, from legumes compared to monoculture. This was confirmed by the increasing levels of soil DOC and  $\text{NO}_3^-$ -N in this study (Table 1). We can conclude, therefore, that legumes have the potential to mitigate the effects of extreme rainfall on the soil microbial community by improving additional soil nutrients.

#### 4.2. Resilience of soil nematodes to extreme rainfall

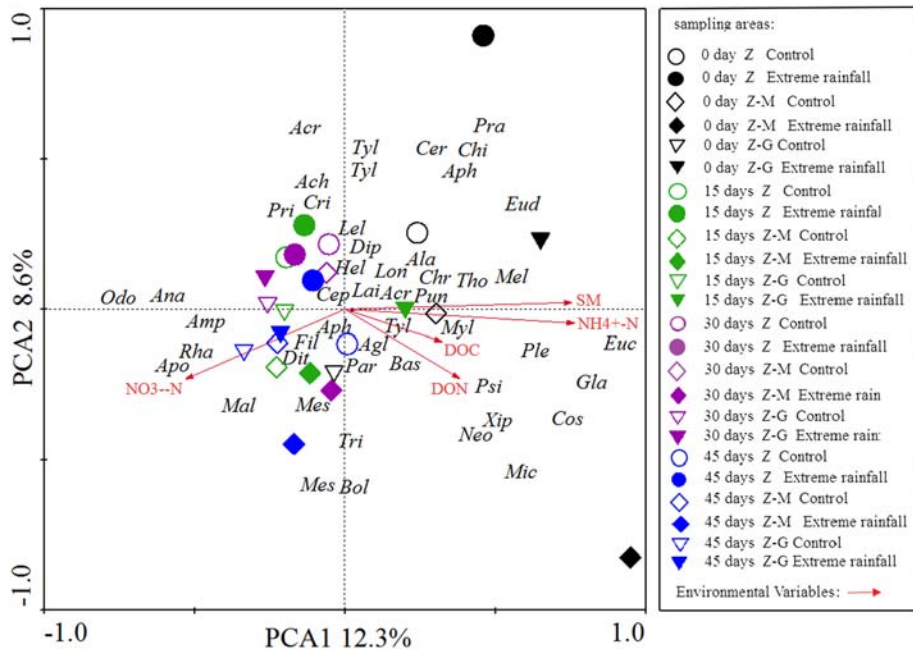
There was a positive correlation between total nematode, bacterivore and omnivore-predator density and soil DOC. This may be due to higher soil DOC content or the dead roots of plants resulting in higher microbial biomass, and that this higher microbial biomass may be the true reason for the higher microbivores. Moreover, plants supply resources, not only to herbivores, but also to microbivores, by releasing large quantities of root exudates (Papatheodorou et al., 2012). This is consistent with a previous study which found that the resilience of bacterivores positively correlated with DOC during drought stress (de Vries et al., 2012). The feeding relationship between nematode and microorganisms may explain why nematodes responded as quickly as microbial populations and were positively correlated with microbial biomass (Thakur et al., 2014). The bottom-up control exerted by trophic-dependencies was a dominant feature of the recovery of soil nematode populations. Root biomass, soil temperature and moisture content have also been identified as important factors affecting plant-feeding nematodes (Verschoor et al., 2001). In our present study, herbivores were positively correlated with soil properties such as DOC and DON. This may be due to plants supplying rich levels of C and N, which then led to an increase in herbivores density. This suggested that plants may indirectly influence plant-feeding nematodes by altering properties of soil.

We observed interesting trends in the trophic groups of nematodes. In monoculture plots, the density of *Thonus* and *Aporcelaimellus* nematodes significantly increased, while the density of *Mesodorylaimus* nematodes decreased. This may be due to the inter-specific competition between *Aporcelaimellus* and *Mesodorylaimus* nematodes, which have the same colonizer-persisters (Table A.1). Thus, nematode genera within the same trophic group responded differently to rainfall stress, and this trade-off resulted in insignificant differences in trophic groups when comparing between extreme rainfall and the control.

Nematode species within the same genera had differing levels of resilience which depended on nematode life history characteristics and the composition of plant species (Shao et al., 2015). In the present study, nematodes belonging to the genus *Rhabditis* recovered, while those belonging to *Odontolaimus* did not recover in the mixed *Z. bungeanum* and *M. sativa* plots. However, both species of nematode recovered in *Z. bungeanum* monoculture and the mixed *Z. bungeanum* and *G. max* plots. This result indicated that the composition, especially the identity, of plant species was more important for the resilience of the soil nematode community.

Soil nematode communities fully recovered to control levels only in *Z. bungeanum* and *G. max* mixed culture. This may be attributed to the higher tolerance to extreme rainfall of *G. max* species, which respond to saturated soil by growing adventitious roots and forming cortex





**Fig. 5.** Principal component analysis of the nematode community composition. Environmental variables are just projected in the graph: Soil water content (SWC), Dissolved organic carbon (DOC), Dissolved organic nitrogen (DON). Abbreviations correspond to the nematode taxa were listed: *Tylenchus* (Tyl), *Malenchus* (Mal), *Psilenchus* (Psi), *Aglenchus* (Agl), *Filenchus* (Fil), *Coslenchus* (Cos), *Lelenchus* (Lel), *Boleodorus* (Bol), *Paratylenchus* (Par), *Neothada* (Neo), *Pratylenchus* (Pra), *Tylenchorhynchus* (Tyl), *Criconemoides* (Cri), *Meloidogyne* (Mel), *Helicotylenchus* (Hel), *Longidorus* (Lon), *Xiphinema* (Xip), *Rhabditis* (Rha), *Mesorhabditis* (Mes), *Odontolaimus* (Odo), *Glaxinema* (Ba1), *Acroboles* (Acr), *Acroboloides* (Acr), *Cephalobus* (Cep), *Eucephalobus* (Euc), *Plectus* (Ple), *Anaplectus* (Ana), *Chiloplacus* (Chi), *Cervidellus* (Cer), *Chronogaster* (Chr), *Bastiania* (Bas), *Prismatolaimus* (Pri), *Alaimus* (Ala), *Amphidelus* (Amp), *Aphelenchoides* (Aph), *Aphelenchus* (Aph), *Ditylenchus* (Dit), *Diphtherophora* (Dip), *Tylencholaimus* (Tyl), *Tripyla* (Tri), *Mylonchulus* (Myl), *Achromadora* (Ach), *Thonus* (Tho), *Eudorylaimus* (Eud), *Microdorylaimus* (Mic), *Pungentus* (Pun), *Mesodorylaimus* (Mes), *Laimydorus* (Lai), *Aporclaimellus* (Apo). Time 0 is the time when extreme rainfall terminated, time 15 is the time after 15 days recovery, time 30 is the time after 30 days recovery, time 45 is the time after 45 days recovery.

**Table 2**  
Pearson correlation analysis between soil properties and soil organisms.

Soil properties	Total microbial biomass	Bacteria biomass	Fungi biomass	Total nematode	Herbivores	Bacterivores	Fungivores	Omnivore-predators
DOC	0.553**	0.530**	0.487**	0.489**	0.370**	0.367**	0.080	0.577**
DON	0.394**	0.374**	0.308**	0.338**	0.289*	0.209	-0.075	0.519**
Soil water content	-0.156	-0.172	-0.232*	0.099	0.431**	-0.095	0.087	0.010
NH <sub>4</sub> <sup>+</sup> -N	0.093	0.062	0.007	0.227	0.413**	0.041	0.109	0.226
NO <sub>3</sub> <sup>-</sup> -N	0.485**	0.467**	0.493**	0.242*	-0.219	0.398**	-0.129	0.373**

The values indicate *r*.  
\* *P* < .05.  
\*\* *P* < .01.

**Table 3**  
Pearson correlation analysis between soil microbial biomass and nematode density.

Soil organisms	Total nematode	Herbivores	Bacterivores	Fungivores	Omnivore-predators
Total microbial biomass	0.514**	0.155	0.532**	0.068	0.570**
Bacterial biomass	0.517**	0.142	0.546**	0.090	0.556**
Fungi biomass	0.535**	0.156	0.568**	0.085	0.556**

The values indicate *r*.  
\*\* *P* < .01.

aerenchyma from roots to stem (Sun et al., 2016), which increases their tolerance to hypoxia tolerance (Tang, 1998). Thus, *G. max* increased the resilience of soil microbial and nematode communities in soil. This result indicated that the composition of plant species had a stronger influence on nematode communities (Viketoft et al., 2009).

Collectively, our current data indicated that the presence of leguminous plant species appeared to increase the resilience of soil nematodes, but this effect depended upon the identity of the plant community. This was consistent with our hypothesis that the

presence of legumes would have a positive effect on the resilience of microbial and nematode communities in soil and that this effect would depend on the actual species of legume involved. The resilience of microbial and nematode communities in soil was clearly coupled with plant composition. Our results indicated that soil nutrients, microbes and nematodes could recover quickly, within only 45 days of an extreme rainfall event, and that the recovery rate depended on plant crop species. However, extreme rainfall did exhibit negative impacts on crop yield, in part, due to negative effects

on soil organisms and nutrients. This finding is important in sustainable agricultural management as selecting suitable associated plants could alleviate the impacts of extreme rainfall.

## 5. Conclusion

The presence of legumes appeared to increase the resilience of the soil microbial community. The resilience of microbial and nematode communities in soil was controlled by bottom-up controls, and also coupled with above-ground plant composition. This study suggested that specific leguminous plants could stabilize the soil food web.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2018.01.063>.

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