Protists and collembolans alter microbial community composition, C dynamics and soil aggregation in simplified consumer - prey systems

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Abstract

Microbes play an essential role in soil functioning including biogeochemical cycling and soil aggregate formation. Yet, a major challenge is to link microbes to higher trophic levels and assess consequences for soil functioning. Here, we aimed to assess how microbial consumers modify microbial community composition (PLFA markers), as well as C dynamics (microbial C use, SOC concentration and CO_2 emission) and soil aggregation. We re-built two simplified soil consumer - prey systems: a bacterial-based system comprising amoebae (*Acanthamoeba castellanii*) feeding on a microbial community dominated by the free-living bacterium *Pseudomonas fluorescens*, and a fungal-based system comprising collembolans (*Heteromurus nitidus*) grazing on a microbial community dominated by the saprotrophic fungus *Chaetomium globosum*. The amoeba *A. castellanii* did not affect microbial biomass and composition, but enhanced the formation of soil aggregates, and tended to reduce their stability. Presumably, the dominance of *P. fluorescens*, able to produce antibiotic toxins in response to the attack by *A. castellanii*, was the main cause of the unchanged microbial community composition, and the release of bacterial extracellular compounds in reaction to predation, such as long-chained polymeric substances or proteases, was responsible for the changes in soil aggregation as side effect. In the fungal system, collembolans significantly modified microbial community composition via consumptive and non-consumptive effects including the transport of microbes on the body surface. As expected, fungal biomass promoted soil aggregation and was reduced in the presence of *H. nitidus*. Remarkably, we also found an unexpected contribution of changes in bacterial community composition to soil aggregation. In both the bacterial and fungal systems, bacterial and fungal communities mainly consumed C from soil organic matter (rather than the litter added). Increased fungal biomass was associated with an increased capture of C from added litter and the presence of collembolans levelled-off this effect. Neither amoebae nor collembolans altered SOC concentrations and CO_2 production. Overall, the reults demonstrated that trophic interactions are important for achieving a mechanistic understanding of biological contributions to soil aggregation

and may occur without major changes in C dynamics, and with or without changes in the composition of the microbial community.

1. Introduction

Soil microbes are essential for soil functioning (Bardgett and Van der Putten, 2014). As major primary decomposers of organic matter, soil microbial communities play a crucial role in soil biogeochemical cycles (Bradford et al., 2013; Graham et al., 2016; Glassman et al., 2018). Microbes as well are key determinants of soil structure, notably soil aggregation (Lehmann et al., 2017). Soil aggregates are "soil-specific entities built from mineral and organic compounds with stronger bonds between building blocks than with neighbouring particles" (Yudina et al., 2019). Soil aggregate properties, in particular their stability, allow to predict large scale soil properties such as soil erosion (Barthes and Roose, 2002). The role of soil microbes on biogeochemical cycles and soil structure has been intensively studied in the past decades (Wieder et al., 2013; Lehmann et al., 2017; Malik et al., 2018). Yet, a major challenge is to link the soil microbial level to higher trophic levels (Grandy et al., 2016). To do so, it is required to investigate how soil microbial grazers and predators impact the composition of the soil microbial community (Thakur and Geisen, 2019), and assess consequences for soil functioning.

Soil microbes form highly diverse and interconnected communities (Delgado-Baquerizo et al., 2016; de Vries et al., 2018; Waag et al., 2019), with bacteria and fungi as key players. In the last decade, increasing effort has been undertaken to decipher biotic interactions within microbial communities (Velicer and Vos, 2009; Schmidt et al., 2015; Deveau et al., 2018), and ultimately link microbial diversity and connectivity to soil biogeochemical cycles (Waag et al., 2019). Bacteria and fungi interact in many ways, with numerous examples of competition and facilitation (Tiunov and Scheu, 2005; Velicer and Vos, 2009; Worrich et al., 2017; Braham et al., 2018), often resulting in complementary actions favouring biogeochemical cycles (Deveau et al., 2018; Waag et al., 2019). However, we know little about how the diversity and interactions within soil microbial communities drive soil structure. So far, general mechanisms underlying effects of bacteria and fungi on soil aggregation have been described using isolated microbial strains, generally pinpointing positive effects (Lehmann et al., 2017). Bacteria influence soil aggregation mainly through the production of extracellular polymeric substances (EPS), which adsorb onto mineral surfaces, increase the viscosity of the soil solution and enhance soil particle cohesion (Chenu, 1993; Sundhya and Ali, 2009; Liu et al., 2013). Filamentous fungi promote soil aggregation in various ways including (i) the enmeshment of soil particles (Degens et al., 1996; Tisdall et al., 1997; Baldock, 2002), (ii) the secretion of polymeric substances (Chenu, 1989; Caesar-TonThat and Cochran, 2000; Daynes et al., 2012), enhancing mineral particle cohesion similar to bacterial EPS, and (iii) the release of a variety molecules, such as hydrophobins (Linder et al., 2005; Zheng et al., 2016) and phenolic acids (Caesar-Tonthat and Cochran, 2000) reducing wettability and preventing soil aggregates from collapsing when the soil is rewetted. However, effects vary between microbial strains. For example, good aggregators were reported for species of the bacterial genera *Bacillus* and *Pseudomonas* (Caesar-TonThat et al., 2007; Caesar-TonThat et al., 2014), and of the fungal

genera *Chaetomium*, *Mucor* and *Aspergillus* (Swaby, 1949). Enhanced aggregative ability was related to higher lipid and protein content in bacterial EPS (Liu et al., 2013), and to higher density of mycelial growth (Lehmann et al., 2020). Such developments with a microbial focus bring us closer to understanding how the diversity of microbes drive biogeochemical cycles and soil aggregation, but fail to connect how these processes are modified in the presence of higher trophic levels.

Soil microbial communities have long been thought to be mainly structured by abiotic filters (Griffiths et al., 2011) and plant inputs (bottom-up regulation; Myers et al., 2001), whereas the importance of higher trophic levels is only yet seen as major, but understudied factor (Thakur and Geisen, 2019) of crucial importance for C cycling (Crowther et al., 2015; Filser et al., 2016; Grandy et al., 2016; Morriën et al., 2017). Several soil organisms can consume bacteria and fungi, including protists, nematodes and collembolans. These consumers often modify the biomass and the composition of the microbial community (Rønn et al., 2002; Djigal et al., 2004; Rosenberg et al., 2009; Perez et al., 2013; Jiang et al., 2017; Geisen et al., 2018; Coulibaly et al., 2019). Such modifications are thought to directly result from consumptive effects, i.e. selective feeding, or indirectly from changes in the competition between microbes due to the consumption of certain microbial strains (Thakur and Geisen, 2019). However, other non-consumptive effects are also at stake (Bradford et al., 2016) as indicated by experimental evidence. For example, the release of N by protists and nematodes (Griffith and Bardgett, 1997), the excretion of urine or faeces by microarthropods (Cragg and Bardgett, 2001; Milcu et al., 2006), as well their necromass (Coleman et al., 2002) modify the quality of organic matter processed by soil microorganisms, with expected effects on their biomass and composition. The comminution of plant debris (Lussenhopp, 1992) as well as the transport of microbes on the body of soil microarthropods (Griffith and Bardgett, 1997; Bardgett et al., 1998; Gormsen et al., 2004) also are assumed to contribute to non-consumptive effects of microarthropods on soil microbial communities. Further, consumers of soil microorganisms can modify microbial activity and physiology, such as the production of bacterial EPS (Matz and Kjellberg, 2005; Queck et al. 2006) and antibiotic compounds (Jousset and Bonkowski, 2010). Variations in enzymatic activity of wood decomposing fungi in the presence of microbivores has also been reported (Crowther et al., 2015). Overall, the influence of trophic interactions on the structure and activity of soil microbial communities is an active research topic (Jiang et al., 2017; Thakur and Geisen, 2019; Lucas et al., 2020). By contrast, the integration of higher trophic levels into soil aggregation studies lag far behind, with only one study investigating how collembolans modify the effect of fungi on soil aggregation (Siddiky et al., 2012) and no studies targeting at effects of protists or nematodes. However, given the importance of microbes for soil aggregation, modifications in microbial community composition or activity due to microbial consumers are expected to play a significant role.

Here, we aimed at investigating how microbial consumers modify microbial community composition, and how this impacts C dynamics and soil aggregation. We focused on two simplified consumer - prey systems: a bacterial- and a fungal-based system, representing the two main, and often interconnected, channels of C fluxes in soil (de Vries and Caruso, 2016). The bacterial-based system comprised the free-living amoeba *Acanthamoeba castellanii* feeding on a microbial community dominated by

bacteria (*Pseudomonas fluorescens* sensu lato), and the fungal-based system comprising the collembolan species *Heteromurus nitidus* grazing on a microbial community dominated by saprotrophic fungi (*Chaetomium globosum*). We conducted a microcosm experiment lasting for 6 weeks and assessed changes in microbial community composition, C dynamics and soil aggregation.

2. Material and methods

2.1 Experimental design and microcosm incubation

We re-constructed simplified bacterial-based and fungal-based prey - consumers systems in soil microcosms. The soil was autoclaved (2 h at 121°C) to reduce and level-off initial microbial abundance and diversity, and re-inoculated with single common soil bacterial or saprotrophic fungal strains, i.e. *P. fluorescens* and *C. globosum*, to set up a bacterial- and a fungal-dominated system, respectively. The resulting microbial community established from the introduced species as well as species that survived autoclaving and those introduced together with the consumers, notably the microbes used to culture the protists and collembolans in the laboratory (*Escherichia coli* and *Saccharomyces cerevisiae*, respectively). These microbial communities formed the prey of the consumers added. The consumers, i.e. the protist *A. castellanii* and collembolan *H. nitidus*, were added two weeks after the microbial inoculation to allow the microbial communities to establish. We accounted for the co-addition of microorganisms with the consumers to microcosms by adding *E. coli* and a microbial wash of the collembolans, respectively. In addition, we set up a zero control in which only autoclaved tap water was added (for full experimental design see Table 1). Overall, our experimental design allowed to disentangle the effects of the addition of consumers on the microbial community due to (i) trophic interactions (consumptive and non-consumptive effects) from those related to (ii) the co-addition of microbes with the consumers. Furthermore, we were able to assess how preceding steps of microbial community establishment influenced soil microbial community composition. We linked the changes in microbial community composition due to the preceding inoculation steps and the addition of consumers to C dynamics and soil aggregation.

In the bacterial-based system, we first added *P. fluorescens* (2×10^9 cfu) and two weeks later *A. castellanii* (6×10^8 individuals) to half of the jars inoculated with *P. fluorescens*. In addition, the mucilage producing Gram$^-$ bacterium *E. coli* (6×10^8 individuals; Danese et al., 2002), used to culture the amoebae, was added to the microcosms that did not receive amoebae (for full experimental design see Table 1). Bacteria and amoebae were added as suspension in autoclaved tap water. In the fungal-based system, we first added the saprotrophic fungus *C. globosum* (4 cm^3 of colonized LB agar), and two weeks later the collembolan species *H. nitidus* (30 individuals per microcosm) to half of the jars inoculated with *C. globosum.* In addition, we added a microbial wash of collembolans (240 individuals) to the microcosms which did not receive the collembolans (for full experimental design see Table 1). The microbial wash as well as *C. globosum* were added as suspension in autoclaved tap

water. *H. nitidus* was added using an entomological exhauster. The same amount of medium (autoclaved tap water with or within smashed LB agar) was added to the respective control treatments.

The bacterium *P. fluorescens* and the fungus *C. globosum* were chosen as they are abundant in soils (Dubuis et al., 2007; Domsch et al., 1993) and beneficially affect soil aggregation (Swaby, 1949; Caesar-TonThat et al. 2014; Tisdall et al., 2012). The amoeba species *A. castellanii* was selected as representative and ubiquitous soil-dwelling amoeba able to consume a broad range of microorganisms and for its ability to produce proteases (Serrano-Luna et al., 2006; Weekers et al. 1995) able to perforate bacterial biofilms essential for soil aggregation. The amoeba species *A. castellanii* was shown to feed on our model strain *P. fluorescens* (Jousset et al. 2009), but prefers less toxic strains. We thus expected that the amoebae will not only feed on *P. fluorescens*, but also on the other bacterial strains of the system (remaining microbial background and *E. coli*). The collembolan species *H. nitidus* was chosen because of its abundance in European temperate soils (Hopkin, 1997) and as it has been shown to preferentially feed on *C. globosum* (Pollierer et al., 2019); feeding on *C. globosum* it is able to survive and reproduce. *H. nitidus* also feeds on bacteria including *P. fluorescens*, but cannot reproduce when feeding only this bacterial species (Pollierer et al. 2019). In our system, we thus expected that *H. nitidus* will mainly feed on *C. globosum*, but will also ingest bacteria present in the microcosms.

The soil used in the experiment comprised a mixture of sand (59.7%) and agricultural soil (39.8%), collected from a wheat agricultural experimental field of the University of Göttingen, managed under conventional tillage and located in the metropolitan area of Göttingen. The agricultural soil was crushed through a 1 mm sieve to destroy larger aggregates and then mixed with sand. The properties of the soil mixture were: 6.0% clay, 30.8% silt, 13.7% fine sand (63-200 µm), 41.4% medium sand (200-630 µm), 8.1% coarse sand (630-2000 µm), 4.5% $CaCO_3$ and 0.36% organic carbon (analyses were conducted by LUFA, Speyer, Germany, following the methods VDLUFA I, C2.2.1:2012 and DIN ISO 10694:1996-08). A total of 120 g dry weight of soil was added per microcosm.

To analyse whether the use of soil C was influenced by consumer - prey interactions, we added a source of particulate organic matter, i.e. dried chopped litter (µm to mm pieces; 0.4%) to the soil mixture. The litter was composed of a mixture of maize (85.3%) and wheat litter (14.7 %). Litter of maize as C4 plant was chosen to allow tracing the incorporation of litter carbon into microorganisms. The organic carbon present in the agricultural soil mainly derived from the residues of wheat as C3 plant.

The microcosms consisted of glass jars (7.5 cm diameter and 10 cm high) containing the mixture of soil, sand and litter (120 g dry weight of soil mixture with litter) and were covered by non-sealed lids, allowing gas and water exchange, but limiting potential contamination in our semi-sterile experimental design. Microcosms were incubated in darkness in a climate chamber at 20°C for a total of 6 weeks (July – August 2017), with the first 2 weeks with microbial prey only. Soil water holding capacity was adjusted weekly to 60% of the maximum by adding autoclaved tap water under sterile conditions.

2.2 Microbial community composition

Changes in soil microbial abundance and composition were quantified by phospholipid fatty acid (PLFA) analysis. Lipids were extracted from fresh soil equivalent to 3.5 g dry weight. The soil was frozen at -20ºC after the experiment until further use according to the protocol of Buyer at al. (2012). PLFAs were measured and identified as described in Pollierer et al. (2015) using a gas chromatograph (GC; Clarus 500 with Autosampler, Perkin Elmer, USA). The mass (nmol g^{-1} of dry soil) of all extracted and identified PLFAs was used as measure of microbial biomass. The PLFA 18:2ω6,9 was used as fungal biomarker and eight PLFAs were used as bacterial biomarkers: i15:0, a15:0, i16:0; i17:0 ($Gram^+$ bacteria), cy17:0, 18:1ω7 ($Gram^-$ bacteria), 16:1ω7 and 15:0 (general bacterial marker) (Frostegård & Bååth 1996; Contosta et al. 2015).

2.3 Microbial C use, CO_2 emission and SOC concentration

The use of C originating from soil (organic C originally present in the wheat agricultural soil) vs. litter (added chopped litter mainly derived from maize; see below) by soil microbes was assessed by tracing the ^{13}C signal of the litter and soil in the microbial PLFA markers. In particular, we investigated the ^{13}C of the fungal PLFA marker and of the eight bacterial PLFA markers, which allowed us to trace the C source of fungal and bacterial communities. The $^{13}C/^{12}C$ ratios of the PLFAs were measured using a trace gas chromatograph (GC; Thermo Finnigan, Bremen, Germany), equipped with a DB5-DB1 column (30 m and 15 m, both 0.25 µm ID, Agilent), and coupled via a GP interface to a Delta Plus mass spectrometer (Thermo Finnigan, Bremen, Germany). The temperature program was run according to the following steps: 1 min at 80˚C, an increase to 170˚C at a rate of 10˚C/min, an increase to 192°C at a rate of 0.7°C/min, 4 min at 192°C, an increase to 200°C at a rate of 0.7°C/min, an increase to 210°C at a rate of 1.5°C/min, a final increase to 300°C at a rate of 10°C/min, and a final step at 300°C during 10 min. Helium was used as carrier gas for injections (250°C). PLFAs were identified by comparison of their chromatographic retention times with those of standard mixtures composed of 37 different FAMEs (Fatty Acid Methyl Esters; Sigma Aldrich, St Louis, USA) ranging from C11 to C24 and 26 BAMEs (Bacterial Fatty Acid Methyl Esters; Sigma Aldrich, St Louis, USA). Isotope ratios were expressed vs. Vienna Pee Dee Belemnite standard (V-PDB) as $\delta^{13}C$ [‰] = $((^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard} - 1) * 1000$. To compare $^{13}C/^{12}C$ ratios of microbial PLFAs to the $^{13}C/^{12}C$ ratios of C sources (organic soil carbon and added litter), we also measured the $^{13}C/^{12}C$ ratios of the agricultural soil used in the microcosms and of the added litter using a Delta Plus mass spectrometer (Thermo Finnigan, Bremen, Germany). Aliquots of ca. 2 g of soil and litter samples were dried (70°C, 24 h), milled (45 s, frequency: 25/s; MM200, Retsch GmbH, Haan, Germany) and placed in a desiccator for 48 h. Aliquots of ca. 25 mg of soil and 0.7 mg of litter were analysed (eight replicates each). The difference between the average isotopic signature of the soil ($\delta^{13}C_{Soil}$ = -27.16 ± 0.06 ‰) and the litter ($\delta^{13}C_{litter}$ = -13.71 ± 0.06 ‰) covered an amplitude of 13.45 ‰ and set the full range of isotopic variation (100 %) used to trace C origin. Using these values as endpoints the proportion of C of soil origin in fungal and bacterial PLFA marker FAs (x) was calculated as follows: % C_{soil} (x) = $[(\delta^{13}C_x - \delta^{13}C_{soil}) \times 100) / (\delta^{13}C_{litter} - \delta^{13}C_{soil})]$ with $\delta^{13}C_x$ the $\delta^{13}C$ signature of the respective marker FA. For bacterial markers, we used average $\delta^{13}C$ values weighted by the relative proportion of each bacterial marker considered. Using the mean

$\delta^{13}C$ value of litter in this calculation assumes that carbon from maize and wheat litter in the litter mixture was used indiscriminately, which we assume to be justified considering the short period of time when predominantly labile litter compounds are used. Further, considering that the litter mixture comprised predominantly maize litter differential incorporation of carbon from wheat is likely to affect the calculations only little. As local hotspots of soil can show lower $\delta^{13}C$ signal than the average $\delta^{13}C$ signature of homogenised soil, the calculation may result in an estimated percentage of C of soil origin higher than 100 %.

To monitor the activity of soil organisms (soil microbiota and soil animals), CO_2 production was measured twice during the incubation period by titration with HCl of 2 mL KOH placed in the jar for 48 h (Fig. 4), following the protocol of Macfadyen (1970). Finally, to estimate whether the inoculation treatments modified the soil organic carbon (SOC) content over the incubation period, SOC concentration of aliquots of soil from the treatments was measured after the end of the incubation period (Fig. 3). Aliquots (ca. 500 mg) of dried (105°C, 24 h) and milled (45 s, frequency: 25/s; MM200, Retsch GmbH, Haan, Germany) soils samples were placed at 600°C during 2 h to remove the organic carbon by combustion. Aliquots of 20 mg of milled soil (unburned and burned at 600°C) were used to measure their carbon content (Vario EL, Elementar, Hanau, Germany), which represented the inorganic and organic carbon, respectively. The soil organic carbon content was obtained by the difference in the concentrations between the total organic carbon and the inorganic carbon.

2.5 Soil aggregate properties

Soil aggregate formation was assessed by soil dry sieving (6 helicoidal movements; 30 cm amplitude) of air-dried (ca. 22°C; 7 days) soil samples using the following sieves: 10 mm, 5 mm, 3 mm, 2 mm, 250 µm and 50 µm, resulting in seven diameter classes of aggregates. As soil was crushed through a 1 mm sieve during soil preparation for microcosm incubation, aggregates larger than 1 mm indicate enhanced cohesion acquired during incubation. The term "aggregate formation" is used to describe the amount and size of aggregates obtained by dry sieving after incubation. Soil aggregate stability was measured following ISO/FDIS (E) 10930 (2012) described in Le Bissonnais (1996) and Le Bissonnais and Arrouays (1997). Briefly, 8 g of dried (40°C, 24 h) soil aggregates (3 – 5 mm) were gently re-wetted by capillarity for 5 min on a buffer paper placed on a saturated sponge. Aggregates then were transferred into ethanol and aggregates > 50 µm were retrieved by sieving in ethanol. The aggregate fraction > 50 µm was dried at 40°C for 24 h and sieved using six sieves (2 mm, 1 mm, 500 µm, 200 µm, 100 µm, 50 µm), resulting in seven diameter classes of aggregates. The mean weight diameter (MWD) was calculated as the average diameter of aggregates weighted by the mass proportion of aggregates within each fraction. The MWD of the dry distribution of aggregates, indicating aggregate formation, is noted MWD_{dd}. The MWD obtained after dispersion of aggregate by gently rewetting, indicating aggregate stability, is noted MWD_{as}.

2.6 Data analyses

Differences between treatments in the concentration of microbial PLFA markers, microbial C use, CO_2 emissions, SOC concentrations and degree of soil aggregation were inspected using generalized least square (GLS) models, followed by ANOVA and post-hoc Tukey tests. Differences in variance between treatments were accounted for in the GLS models. The effects of treatments on bacterial PLFA composition (8 specific markers) were investigated using non-metric multidimensional scaling (NMDS), followed by discriminant function analysis (DFA). Overall differences in bacterial PLFA composition within the bacterial and fungal system first were analysed by MANOVA. Pairwise differences between treatments were further inspected using Mahalanobis distances. Changes in soil bacterial community composition were captured by the scores (Axis 1) of principal component analyses (PCA) of the eight bacterial PLFA markers for further analyses. Axis 1 of the PCA on bacterial markers of the bacterial and fungal system explained 39.3 % and 37.6 % of total variability, respectively. Pairwise correlations among (i) descriptors of soil microbial community and (ii) soil aggregation and C dynamics were tested using Spearman's correlation coefficients (ρ). Best explanatory variables of soil aggregation, microbial C use, SOC concentrations and CO_2 production were identified among the descriptors of the microbial community composition using simple linear generalized regression models (glm) and selected according to their R^2 and ΔAIC. Only significant models are displayed. All statistical analyses were run separately for the bacterial and fungal systems. Data provided in the text represent means ± standard deviation. All statistical analyses were conducted in R version 3.6.1 (R Development Core Team, 2008).

3. Results

3.1 Microbial community composition as affected by consumer - prey system

Overall, consumer - prey inoculation had little influence on soil microbial biomass, but modified the composition of microbial communities (Fig. 1). In the bacterial system, the predator *A. castellanii* did not significantly affect total microbial biomass, fungal-to-bacterial (F : B) PLFA ratio, bacterial community composition and the proportions of $Gram^+$ and $Gram^-$ bacteria (Figs 1, 2, Table 2). In the fungal system, the grazer *H. nitidus* significantly decreased fungal biomass, as well as the F : B ratio, but did not induce changes in microbial and bacterial biomass, or in the proportion of $Gram^+$ and $Gram^-$ bacteria (Fig. 1). Despite this lack of changes in specific bacterial markers, the addition of collembolans influenced the overall composition of the bacterial community (pairwise comparison, NMDS/DFA/Mahalanobsis distances; F = 4.8, *P* = 0.03, Fig. 2, Table 2). Initial steps of microbial community establishment also significantly affected microbial community composition. In the bacterial system, treatments where *E. coli* and *P. fluorescens* were added showed significantly higher proportions of $Gram^-$ bacteria, and lower proportions of $Gram^+$ bacteria (Fig. 1 E-G). Total bacterial biomass was lowest in both of these treatments (Fig. 1 C). Further, the addition of *P. fluorescens* significantly modified the bacterial community composition (pairwise comparison, NMDS/DFA/Mahalanobsis distances; F = 15, *P* = 0.0002, Fig. 2, Table 2), whereas the addition of *E. coli* had no significant effect (pairwise comparison, NMDS/DFA/Mahalanobsis distances; F = 5.2, *P* = 0.05, Fig. 2, Table 2). In the fungal system, the inoculation of the microbial wash did not affect fungal and bacterial biomass, or the proportions of $Gram^+$ and

Gram⁻ bacteria (Fig. 1). However, the microbial wash significantly modified the overall bacterial community composition (pairwise comparison, NMDS/DFA/Mahalanobsis distances; F = 28.0, $P < 0.0001$, Fig. 2, Table 2). The inoculation of *C. globosum* significantly increased fungal biomass and decreased bacterial biomass, which led to an overall increase in the F : B ratio (Fig. 1 B-D). Treatments where *C. globosum* was added showed a significantly lower proportion of Gram⁻ bacteria and the addition of *C. globosum* significantly modified the bacterial community composition (NMDS/DFA/Mahalanobsis distances; F = 11.0, $P = 0.002$). Overall, the bacterial community composition was significantly modified by every step of the establishment of the prey - consumer fungal-based system as indicated by the significant differences between all pairs of treatments (Fig. 2, Table 2).

3.2 Microbial C use, CO_2 emissions and SOC concentrations as affected by consumer - prey system

Based on ^{13}C content in microbial PLFA markers, most of the bacterial (73 ± 14 % across all treatments) and fungal C (64 ± 22 %) originated from soil C (rather than litter C). In the bacterial systems these figures did not vary significantly among treatments (Fig. 3). The addition of the amoeba *A. castellanii* thus did not modify the use of C sources. In the fungal system, the origin of fungal and bacterial C differed. In microcosms with fungi only, 52 ± 11 % of fungal C originated from soil, whereas 72 ± 4.3 % of bacterial C was of soil origin, indicating that fungi captured more litter C (48 ± 11 %) than bacteria (28 ± 4.3 %). These differences levelled off in the presence of collembolans (Fig. 3 A), indicating that the addition of *H. nitidus* modified the use of C sources by soil microbes. Despite these differences between fungi and bacteria in their ability to capture C resources, no significant differences were observed in terms of CO_2 emitted and SOC concentrations between treatments (Fig. 3 B, C).

3.3 Soil aggregation as affected by consumer - prey system

The 6 week incubation period in microcosms resulted in the formation of soil aggregates, regardless of the treatments (mean MWD_{dd} across all treatments = 3.84 ± 0.8 mm), compared to the initial soil conditions (MWD_{dd} = 0.77 ± 0.01 mm). The formed aggregates on average were unstable (mean MWD_{as} across all treatments = 0.58 ± 0.15 mm) and ranged from very unstable (min. MWD_{as}= 0.31 mm) to moderately stable (max. MWD_{as}= 1.04 mm), according to the classification of the international norm ISO/FDIS10930 (E) (2012). The protist and collembolan consumers had contrasting effects on soil aggregate formation. Adding *A. castellanii* significantly increased the formation of soil aggregates, whereas *H. nitidus* significantly decreased it (Fig. 4 A, B). Effects of consumers on soil aggregate stability were weaker but more consistent in the bacterial than in the fungal system. Both *A. castellanii* and *H. nitidus* tended to reduce the stability of soil aggregates (Fig. 4 A, B). These effects were weak as no significant differences were observed by direct comparison of the treatments with *P. fluorescens* or *C. globosum* with and without their respective consumers. However, the significant increase in soil aggregate stability in response to the addition of *P. fluorescens* and *C. globosum* vanished when their associated consumers were added. Overall, this indicates that *A. castellanii* and *H. nitidus* reduce the positive effect of *P. fluorescens* and *C. globosum* on soil aggregate stability,

although only little. The initial inoculation of soil microorganisms also modified the formation and stability of soil aggregates. In particular, *P. fluorescens* increased the stability of the soil aggregates, but did not modify their formation (Fig. 4 A, B). *C. globosum* had a significant positive effect on both soil aggregate formation and stability (Fig. 4 A, B). Neither the addition of *E. coli* nor the microbial wash significantly modified soil aggregation.

3.4 Relationships between microbial community composition, C dynamics and soil aggregation

Descriptors of the microbial community composition were poorly related among each other in the bacterial system, whereas in the fungal system they were more correlated (Fig. 5, Table A1). In the bacterial system, bacterial biomass positively correlated with the ratio of $Gram^+$ to $Gram^-$ bacteria ($\rho = 0.58$, $P = 0.0007$). These two variables also were significantly related to the microbial community composition (as indicated by PCA scores, $P < 0.0001$). Despite overall low levels of fungal biomass in the bacterial system, fungal biomass positively correlated with the F : B ratio ($\rho = 0.78$, $P < 0.0001$), which itself positively correlated with microbial biomass ($\rho = 0.39$, $P = 0.03$). In the fungal system, fungal biomass and the F : B ratio (themselves positively related, $\rho = 0.99$, $P < 0.0001$) were positively correlated with microbial biomass ($P = 0.003$ and $P = 0.002$, respectively). Fungal biomass and F : B ratio also significantly correlated with the bacterial community composition (as indicated by PCA scores, $P < 0.0001$). The composition of the bacterial community was significantly related to the $Gram^+$ to $Gram^-$ bacteria ratio ($\rho = 0.71$, $P < 0.0001$), itself being related to fungal biomass ($\rho = 0.39$, $P = 0.04$), but not to the F : B ratio ($\rho = 0.29$, $P = 0.13$). Fungal biomass negatively correlated with bacterial biomass ($\rho = -0.56$, $P = 0.002$).

In both the bacterial and fungal system, soil aggregation, microbial C use, CO_2 emissions and SOC concentrations were poorly correlated (Fig. 5, Table A2). In the bacterial system, no correlations were found between soil aggregate formation, soil aggregate stability, bacterial C use, CO_2 emissions and SOC concentrations (Fig. 5 A). In the fungal system, soil aggregate formation and stability were positively correlated ($\rho = 0.46$, $P = 0.009$). In addition, fungal C use positively correlated with bacterial C use ($\rho = 0.59$, $P = 0.003$), indicating that communities characterized by higher capture of litter C by fungi also presented higher incorporation of litter C into bacteria. SOC concentrations and CO_2 emissions were not related to each other, neither to microbial C use nor soil aggregation.

Variations in soil aggregation and C dynamics were not explained by any of the descriptors of the microbial community in the bacterial system (Fig. 5, Table A3). In the fungal system, some microbial community parameters were related to soil aggregation and overall C use (single generalized linear models). In particular, soil aggregate formation and stability both positively correlated with fungal biomass ($P = 0.002$ and 0.007, respectively; Table A3) and the F : B ratio ($P = 0.004$ and 0.02, respectively; Table A3), which were strongly correlated variables (Table A2). Beside the dominant effect of fungal abundance (R^2 and AIC criteria), soil aggregate formation was negatively related to bacterial biomass ($R^2 = 0.18$, $P = 0.02$), but this was less strong according to R^2 and AIC criteria (Fig. 5 B, Table A3). Concerning soil aggregate stability, the effects of fungal abundance, i.e. fungal biomass and F : B ratio, came second and third, respectively, following the effect of the

bacterial community composition (R^2 and AIC criteria), which significantly correlated with soil aggregate stability (R^2 = 0.28, P = 0.002; Table A3). In addition, the Gram$^+$ to Gram$^-$ bacteria ratio positively correlated with soil aggregate stability, but less strong (R^2 = 0.16, P = 0.03, higher AIC). Fungal C use positively correlated with bacterial biomass (R^2 = 0.22, P = 0.02) in the fungal system, indicating that fungi acquired more soil C when bacterial biomass was increased. The production of CO_2 and the SOC concentration did not correlate with any microbial parameters in both the bacterial and fungal system.

4. Discussion

Our results showed that simplified trophic interactions modified microbial community composition and soil aggregation, but did not or only little affect C dynamics. Overall, the effects were stronger in the fungal-based system than in the bacterial-based system. In the latter, the inoculation of *P. fluorescens* as dominant bacterial strain in large drove the changes in microbial community composition, whereas the addition of the amoeba predator *A. castellanii* did not induced further changes, presumably because *P. fluorescens* is a less preferred and toxic strain for *A. castellanii*. However, *A. castellanii* enhanced the formation of soil aggregates, presumably related to changes in the production of bacterial EPS in response to the attack by *A. castellanii*. In the fungal-based system, conform to our expectations, the inoculation of *C. globosum* increased fungal biomass and the addition of the grazer *H. nitidus* reduced it. These variations in fungal biomass were positively related to changes in soil aggregation, suggesting a detrimental effect of collembolans on soil aggregation. Surprisingly, the inoculation of *C. globosum* and *H. nitidus* resulted in significantly modified bacterial biomass and composition, and this was related to changes in soil aggregation. Finally, in the bacterial- and fungal-based systems, soil organic matter was the dominant C source and inoculation steps only weakly modified the relative importance of soil vs. added chopped litter as microbial C source. Notably, the inoculation treatments did not significantly affect SOC concentrations and CO_2 emissions, suggesting that despite trophic interactions significantly modify microbial communities and soil aggregation this may not change soil C dynamics.

4.1 Bacteria-based prey - consumer system

The composition of the microbial community was mainly driven by the addition of *P. fluorescens*, which dominated the system and is a less preferred toxic prey for *A. castellanii* (Jousset et al., 2009). As *P. fluorescens* is a Gram$^-$ bacterium, the increased proportion of Gram$^-$ bacteria in the system after its addition was expected. Interestingly, the addition of *P. fluorescens* also decreased the proportion of Gram$^+$ bacteria as well as overall bacterial biomass. Thus, *P. fluorescens* presumably constrained the growth of the remaining microbial background community present in the system, notably by outcompeting Gram$^+$ bacteria (Powers et al., 2015).

The addition of the amoeba *A. castellanii* overall weakly affected bacterial abundance and community composition. This lack of effect contrasts previous work reporting a strong decrease in bacterial biomass as well as significant and rapid changes in bacterial community composition in presence of *A. castellanii* (Jousset et al., 2009; Rosenberg et al., 2009). One possible

explanation for this discrepancy is the dominance of *P. fluorescens* and its ability to produce toxins in response to grazing by *A. castellanii* (Jousset and Bonkowski, 2010). In fact, *P. fluorescens* usually is not a preferred prey for *A. castellanii* (Jousset et al., 2009). In our system, the amoebae thus presumably only moderately fed on *P. fluorescens*, and exerted stronger pressure on the other less abundant bacterial strains, namely *E. coli* and the bacteria from the remaining microbial background. As a consequence, there may have been changes in the composition of non-toxic bacterial strains, and in the proportions of $Gram^-$ and $Gram^+$ bacteria, the latter usually being less preferred by protozoa due to their protective cell wall (Ronn et al., 2002, Murase et al., 2006). However, protected from protozoan predation the dominance of *P. fluorescens* masked these changes. In addition, due to preferential feeding on non-toxic strains, one could have expected beneficial effects of amoebae on *P. fluorescens*, but no increase in $Gram^-$ bacteria occurred in treatments with *A. castellanii*. This lack of change may have resulted from several concomitant factors. First, *P. fluorescens* was dominating the bacterial community, leading to little release in the competitive pressure due to preying on the less abundant non-toxic bacteria. Further, low abundance of non-toxic bacterial strains in our system resulted in low nutrient release due to grazing by protists on these bacteria (Jousset et al. 2008). The potential benefit for the growth of *P. fluorescens* thus presumably was minor. Moreover, although *P. fluorescens* is a non-preferred prey it nonetheless is consumed by *A. castellanii* (Jousset and Bonkowski, 2010), and this may have counteracted positive effects on its growth. Finally, the activation of toxin production by *P. fluorescens* is costly (Zha et al., 2006), and the production of such defence molecules usually is associated with reduced investment in bacterial growth (Malik et al., 2020). A more detailed investigation of bacterial community composition using molecular tools may have allowed deeper insight into these processes and should be employed in future studies.

Soil aggregation, but not bacterial C use, SOC concentrations and CO_2 production, was modified by the inoculation of the consumer - prey system. The bacterial community incorporated most of its C from soil (73.4 ± 14.0 %) and this remained unchanged regardless of the inoculation treatment. More homogeneous distribution of soil organic matter, already processed and embedded into the soil matrix, compared to the added chopped litter, composed of relatively large pieces (µm to mm size) of non-processed plant debris, may explain this high incorporation (Malik et al., 2016; Tecon and Or, 2017). The unchanged proportion of C sources by bacteria when specific bacterial strains or the amoeba predator were added is consistent with the lack of effect of these inoculation steps on overall microbial biomass. The unchanged levels in CO_2 emission and SOC concentrations as well were in line with the constant microbial biomass. What was more surprising is that despite microbial biomass and composition as well as C dynamics remained unchanged, we observed modifications in soil aggregation after the amoeba predator was added. To the best of our knowledge, this is the first experimental evidence that protists affect soil aggregation, and that significant effects of amoebae can occur without modifying the biomass and the composition of the microbial community. Possible explanations are expected changes in microbial activity. In our case, *P. fluorescens* was dominant and presumably weakly affected by *A. castellanii* in terms of biomass, but both may have changed their activity. In particular, *P. fluoresencens* responds to predation by *A. castellanii* through enhanced production of phenolic compounds with antibiotic properties (Jousset and Bonkowski, 2010). Enhanced mucilage production, with higher production of

polysaccharides, as well is a common strategy used by bacteria in response to predation by protists, leading to higher bacterial survival and growth (Matz and Kjellberg, 2005; Queck et al. 2006). Further, *A. castellanii* may have upregulated the production of proteases enabling the amoebae to perforate bacterial biofilms (Serrano-Luna et al., 2006). All these compounds released by the amoeba predator and its bacterial prey have a significant potential to induce changes in soil aggregation as side effect. Polysaccharides (Chenu, 1989; Chenu, 1993), proteins (Rillig et al., 2004; Liu et al., 2013; Erktan et al., 2017) and phenolic compounds (Yoshikawa et al., 2018) are all known for their important role in soil aggregation. It is plausible that an enhanced production of mucilage, notably polysaccharides, by *P. fluorescens* may be the underlying cause of the formation of larger soil aggregates after the addition of *A. castellanii*. Furthermore, the release of proteases by *A. castellanii* may have decreased the wettability of the mucilage, and thereby have been responsible for the lack of associated significant increase in the stability of aggregates. Although our focus was not to quantify changes in extracellular compounds occurring in amoeba - bacteria interactions, our results suggest that further investigations in this direction may be promising, and could explain the effects of protists on soil aggregation. Initial steps of the inoculation of the prey - consumer system also influenced soil aggregation. In absence of the amoeba predator, the bacterial communities, activated by wet and dry cycles (Cosentino et al., 2006), promoted the formation of soil aggregates. This effect occurred regardless of the bacterial strains inoculated, suggesting a similar ability of these bacterial strains to increase soil particle cohesion. By contrast, the inoculation of *P. fluorescens* significantly stabilised soil aggregates, suggesting specific hydrophobic properties of its EPS.

Overall, the effects of the amoeba *A. castellanii* on the microbial community were limited, and neither induced changes in microbial biomass and composition nor in C dynamics. However, despite this lack of effects the addition of the amoeba predator significantly affected soil aggregation. Such changes in soil aggregation irrespective of changes in C dynamics and microbial composition suggest that protists preying on bacterial communities can significantly affect soil structure, without significantly affecting microbial growth and C cycling. Presumably, changes in the activity of microbiota, notably the release of extracellular compounds in response to predation by protists, are responsible for changes in soil aggregation as side effects.

4.2 Fungi-based prey - consumer system

The microbial community was influenced by every inoculation step in the fungal-based system including by the addition of the collembolan grazers, and showed strong relations between the fungal and bacterial communities. As expected, fungal biomass was highest in the treatment with *C. globosum*, indicating that our inoculation was successful and triggered fungal development, which drove an overall increase in microbial biomass. Remarkably, the increase in fungal biomass was associated with a decrease in bacterial biomass as well as in $Gram^-$ bacteria, indicating competition between fungi and bacteria as is common in the top soil (Braham et al., 2018). Adding collembolans reduced fungal biomass, presumably due to the consumption of fungal hyphae by *H. nitidus*, confirming that *C. globosum* is consumed by *H. nitidus* (Pollierer et al., 2019). In addition, as collembolans also are feeding on litter (Potapov et al. 2016), competition for litter resources between fungi and collembolans may have contributed to the reduced fungal biomass. Unexpectedly, bacterial biomass did not benefit from the

decrease in fungal biomass due to the addition of *H. nitidus*. This might have been due to (i) the competitive pressure exerted by fungi remained high in the presence of *H. nitidus*, and/or (ii) the potential positive effects of reduced competition were compensated by *H. nitidus* also feeding on bacteria (Pollierer et al., 2019), even though probably at low level. In fact, the composition of the bacterial community was significantly modified by collembolans. Presumably, these changes were due to selective feeding of *H. nitidus* on certain bacterial strains, and/or through modifications in competitive interactions between certain bacterial strains and *C. globosum* (Lussenhopp, 1992; Coulibaly et al., 2019; Thakhur and Geisen, 2019). In addition, other non-consumptive effects may have occurred. For example, comminution of the added litter by collembolans (Coleman et al., 2002) may have contributed to changes in bacterial community composition (Scheu et al., 2005). Further, the release of specific organic compounds by living or dead collembolans, such as urine, faeces (Lussenhop, 1992) or chitin (Lucas et al., 2019), also may have modified the composition of the bacterial community. Notably, the addition of the collembolan wash also significantly modified the bacterial community composition. Although it has long been assumed that microorganisms from the body surface of microarthropods may affect soil microbiota (Lussenhopp, 1992; Scheu et al., 2005), our results provide experimental evidence for it through the separate addition of a microbial wash. This effect highlights the importance of processes of community coalescence (Rillig et al., 2015) caused by the carriage of microorganisms on the body of the consumers. Far from anecdotic, these effects were as strong as those when the soil animals themselves were added, suggesting that future studies should integrate such non-consumptive effects as main driver of the role of microarthropod grazers on soil microbial community composition.

Modifications in the composition of the microbial community were associated by changes in microbial C use and soil aggregation, but not by changes in SOC concentrations and CO_2 emissions. More C of litter origin was captured by fungi rather than bacteria when fungal biomass was high. Superior ability of fungi than bacteria in capturing litter C has been observed previously in microcosm experiments (Malik et al., 2016), and is consistent with the network structure of fungal mycelia able to bridge air-filled pores (Otten et al. 2001), and thus able to reach distant resources such as pieces of litter widely distributed in the soil matrix. In our study, the increased use of litter C by fungi was associated by an increased use of litter C by bacteria. This positive correlation suggests that fungi facilitated the access of litter C to bacteria, confirming previous similar observations (Purahong et al., 2016; Gorka et al., 2019). This positive effect may have been due to the transfer of nutrients by fungal hyphae (Worrich et al., 2017) or increased mobility of bacteria along fungal hyphae (Simon et al. 2015; Worrich et al., 2016). Effects, however, were minor and did not affect the bacterial C source, which remained to be based mainly on soil C. Interestingly, all these changes in microbial biomass, composition and C use occurred without impacting the overall production of CO_2 and SOC concentration. Potentially, concomitant opposite effects of increased microbial biomass and decreased microbial activity have been responsible for the overall constant levels of soil respiration. Such compensatory effects have been proposed previously to be responsible for the absence of effects of higher trophic level consumers on soil respiration (Mikola and Setälä, 1998; Lucas et al., 2020).

The positive effect of *C. globosum* on soil aggregation is consistent with previous studies highlighting positive effects of fungi, in particular saprotrophic ones, on soil aggregation (Caesar-TonThat, 2000; Daynes et al., 2012; Lehmann et al., 2017), especially when fungi dominate the microbial community (McMalla et al., 1958). Collembolans reduced the positive effect of *C. globosum* on soil aggregation. As hypothesized, the reduction in fungal biomass by collembolans presumably was responsible for the negative effect of *H. nitidus* on soil aggregation in our system. More intriguing changes in bacterial community composition related to inoculation steps also contributed to variations in soil aggregate stability. Previous studies reported positive effects of interactions between fungi and bacteria on soil aggregation (Aspiras et al., 1971) as well as differential effects of $Gram^+$ and $Gram^-$ bacteria (Mu'minah et al., 2015). Our results further suggest that changes in the bacterial community composition contribute to a similar extent as fungal biomass to changes in soil aggregate stability. Our design does not allow to disentangle the individual effects of fungal biomass and bacterial community composition, but we suggest that a combination of both was responsible for the observed overall effects on soil aggregate stability. Altogether, we showed that collembolans modified the microbial community composition, presumably through both consumptive and non-consumptive effects, which impacted soil aggregation.

5. Conclusions

We demonstrated that simplified consumer - prey interactions influence soil microbial community composition, microbial C use, soil aggregation, but not CO_2 emissions and SOC concentrations. Remarkably, effects on soil aggregation were not related to SOC or CO_2 emissions, suggesting that subtle but significant effects related to trophic interactions can occur at constant levels of SOC and microbial respiration. In the bacterial-based system, changes in soil aggregation occurred without any modification in microbial biomass and community composition. This lack of change presumably resulted from the dominance of *P. fluorescens*, a toxic and non-preferred prey for *A. castellanii*. We conjecture that changes in bacterial physiology, such as increased EPS production and/or the release of secondary metabolites in response to predation, is the main cause of the formation of larger aggregates. In the fungal-based system, the collembolan species *H. nitidus* significantly modified the microbial community via decreasing the biomass of *C. globosum* and changing bacterial community composition. Notably, we found that the effects of collembolans on the soil microbial community did not only result from consumptive effects, suggesting that the transport of soil microorganisms on the body surface of collembolans is of major importance for the effect of higher trophic levels on soil microbial communities. As expected, fungal biomass was a main factor promoting soil aggregation, and was negatively modulated in the presence of the fungal grazer *H. nitidus*. Remarkably, we also found an unexpected contribution of changes in bacterial community composition on soil aggregation. We conclude that the effects of consumer - prey interactions on soil aggregation can be either positive or negative and were in our case stronger on the formation of soil aggregates than on their stabilisation. Our results highlight that non-trivial changes in microbial community composition and/or activity caused by microbial consumers impact soil aggregation as side effects. Integration of more complex trophic interactions are needed to assess how the mechanisms highlighted in our simplified systems are embedded into more complex processes in natural systems.

6. Data availability

In the case of acceptance of the manuscript, the data supporting the results will be archived in a public repository (Dryad or Zenodo) and the data DOI will be included at the end of the article.

7. Author contribution

AE and SS designed the study. MR, AJ and AC provided advise on the experimental design. AE conducted the microcosms experiment. AJ provided the culture of *Acanthamoebae castellanii*. AE analysed the PLFAs data. AE ran the statistical analyses and wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

8. Competing interests

The authors declare that they have no conflict of interest.

9. Acknowledgements

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Tables

Table 1: Experimental design
Details on the experimental design. X indicate which soil organisms have been added to the systems.

		Bacterial based system			Fungal based system			
Treatment		**Prey**	**Prey and consumer**	**Consumers associated microbiota**	**Prey**	**Prey and Consumer**	**Consumers associated microbiota**	**Common zero control**
Specific bacterial and fungal strains	*Pseudomonas fluorescens*	X	X					
	Chaetomium globosum				X	X		
Consumers	*Acanthamoeba castellanii*		X					
	Heteromorus nitidus					X		
Consumers associated microbiota	*Escherichia coli*	X	(X) Directly added by predator inoculation	X				
	Microbial wash of Collembolan				X	(X) Directly added by grazer inoculation	X	

Table 2 Effect of bacterial and fungal consumer - prey inoculations on bacterial community composition. Differences in the composition of bacterial PLFAs between treatments were analysed using non-metric multidimensional scaling (NMDS), followed by discriminant function analysis (DFA). Overall differences in terms of bacterial PLFA composition (8 markers) within the bacterial and fungal systems were first analysed by MANOVA. Pairwise differences between treatments were further investigated using Mahalanobsis distances. Inoculation treatments are underlined. RMB: remaining microbial background; coll. wash: collembolan wash.

Pair of treatments (a // b)	Statistical parameters		
	F-value	*P-value*	Mahalanobsis distance
Bacterial system			
Overall differences (MANOVA)	14	<0.0001	-
RMB + *E. coli* + *P. fluo* I RMB + *E. coli* + *P. fluo* + *A. castellanii*	2.1	0.21	0.9
RMB + *E. coli* + *P. fluo* I RMB + *E. coli*	15	0.0002	3.2
RMB + *E. coli* + *P. fluo* I RMB	172	<0.0001	4.9
RMB + *E. coli* I RMB + *E. coli* + *P. fluo* + *A. castellanii*	17	0.0001	4.0
RMB I RMB + *E. coli* + *P. fluo* + *A. castellanii*	115	<0.0001	5.1
RMB I RMB + *E. coli*	5.2	0.04	2.7
Fungal system			
Overall differences (MANOVA)	16	<0.0001	-
RMB + coll. wash + *C. globosum* I RMB + coll. wash + *C. globosum* + *H. nitidus*	4.8	0.03	3.2
RMB + coll. wash + *C. globosum* I RMB + coll. Wash	11	0.002	3.5
RMB + coll. wash + *C. globosum* I RMB	42	<0.0001	6.4
RMB + coll. wash I RMB + coll. wash + *C. globosum* + *H. nitidus*	13	0.0008	3.7
RMB I RMB + coll. wash + *C. globosum* + *H. nitidus*	49	<0.0001	5.4
RMB I RMB + coll. wash	28	<0.0001	4.0

Figures

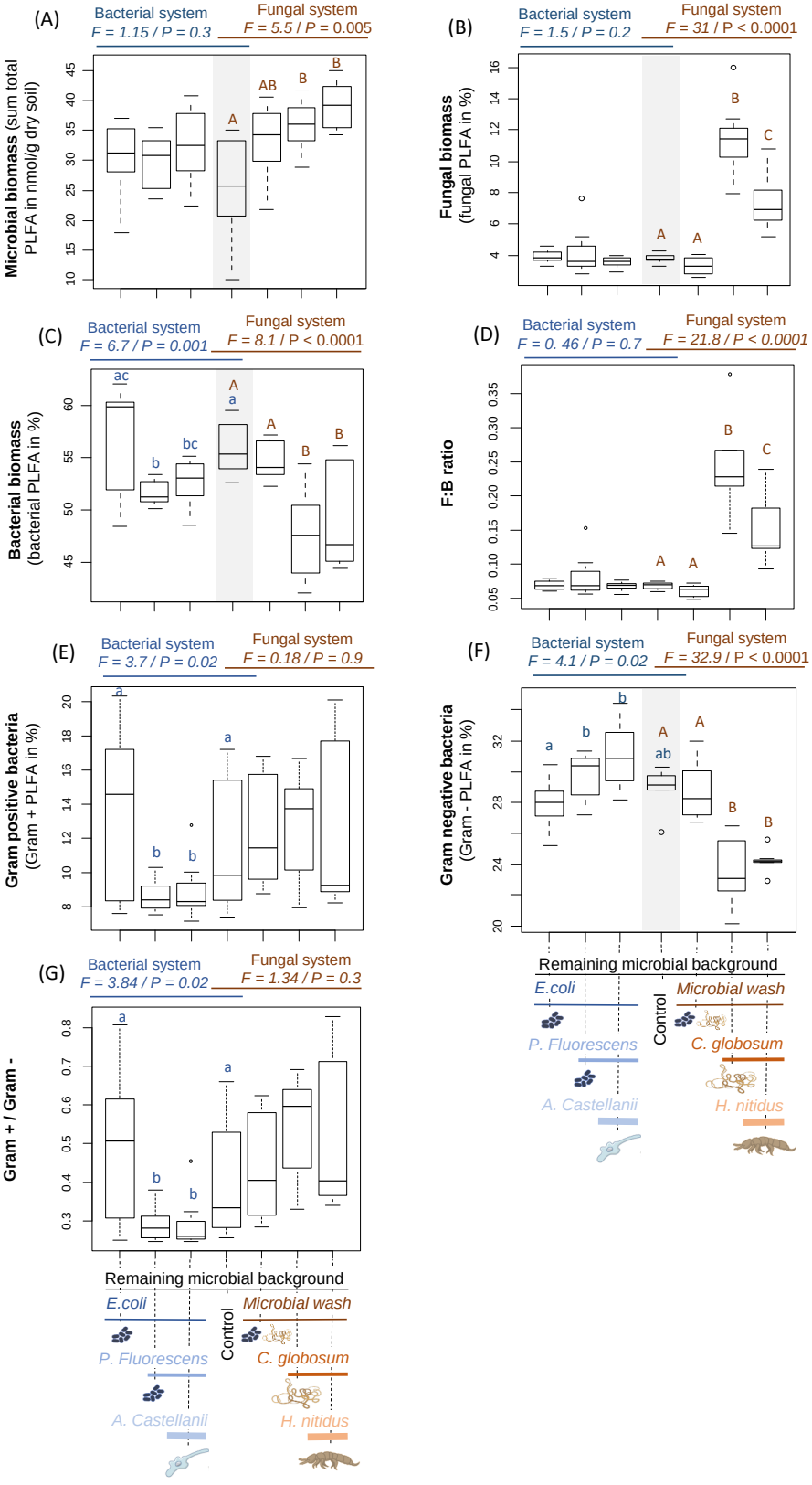
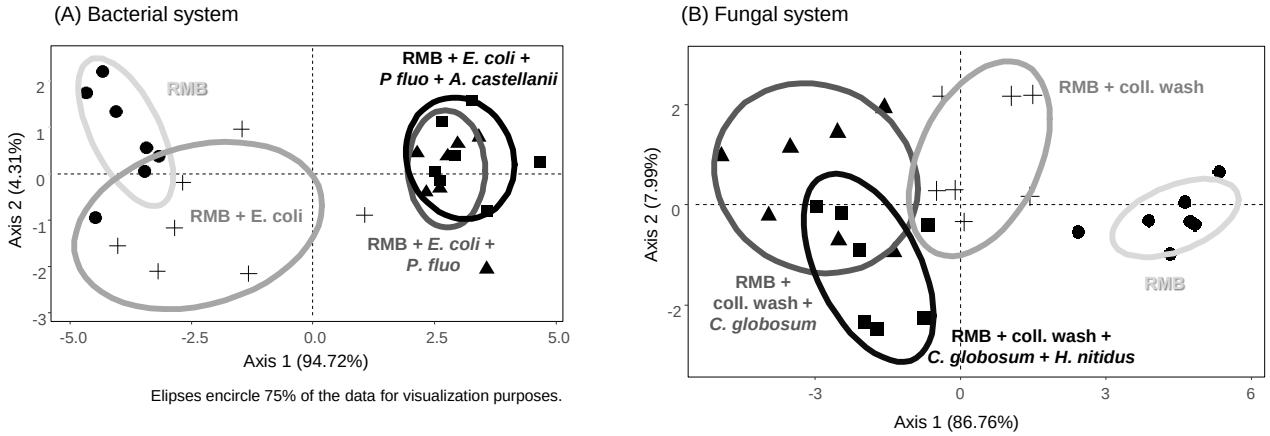
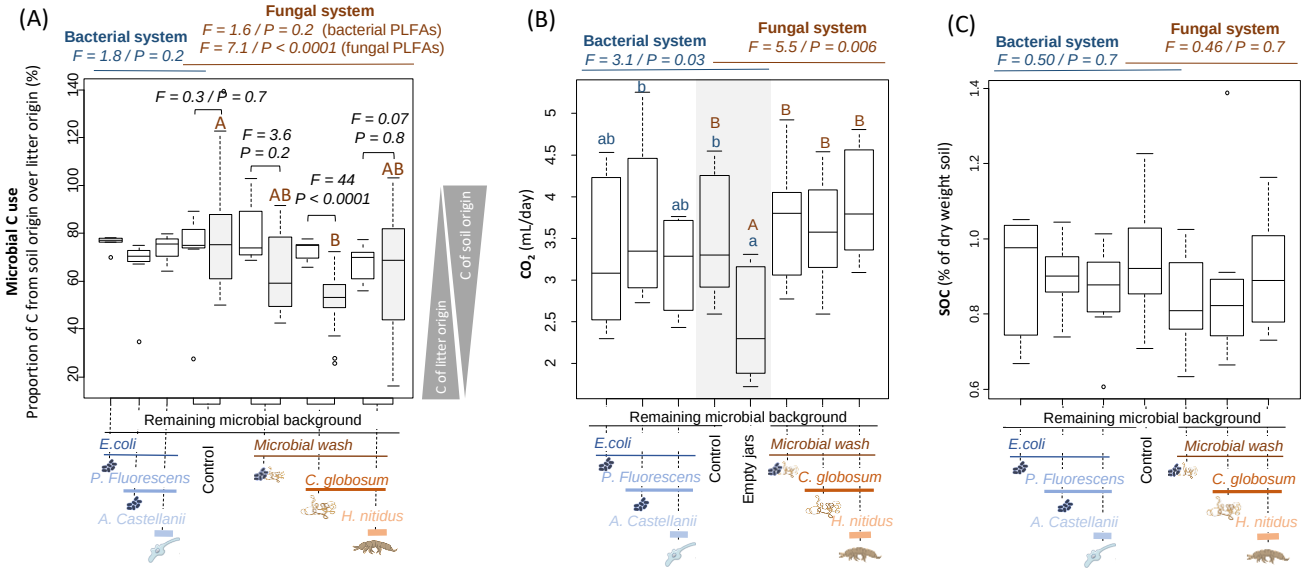

(A)
Bacterial system
F = 1.15 / P = 0.3
Fungal system
F = 5.5 / P = 0.005
Microbial biomass (sum total PLFA in nmol/g dry soil)
(B)
Bacterial system
F = 1.5 / P = 0.2
Fungal system
F = 31 / P < 0.0001
Fungal biomass (fungal PLFA in %)
(C)
Bacterial system
F = 6.7 / P = 0.001
Fungal system
F = 8.1 / P < 0.0001
Bacterial biomass (bacterial PLFA in %)
(D)
Bacterial system
F = 0. 46 / P = 0.7
Fungal system
F = 21.8 / P < 0.0001
F:B ratio
(E)
Bacterial system
F = 3.7 / P = 0.02
Fungal system
F = 0.18 / P = 0.9
Gram positive bacteria (Gram + PLFA in %)
(F)
Bacterial system
F = 4.1 / P = 0.02
Fungal system
F = 32.9 / P < 0.0001
Gram negative bacteria (Gram - PLFA in %)
(G)
Bacterial system
F = 3.84 / P = 0.02
Fungal system
F = 1.34 / P = 0.3
Gram + / Gram -
Remaining microbial background
E.coli
P. Fluorescens
A. Castellanii
Control
Microbial wash
C. globosum
H. nitidus

Figure 1 Effect of bacterial and fungal consumer - prey inoculations on microbial biomass and composition. (A) Microbial biomass, (B) fungal biomass, (C) bacterial biomass, (D) fungal : bacterial PLFA ratio (F : B), (E) proportion of $Gram^+$ bacteria and (F) $Gram^-$ bacteria, and (G) ratio between $Gram^+$ and $Gram^-$ bacteria. Differences between treatments were analysed separately for the bacterial and fungal systems using GLS models followed by ANOVA and post-hoc Tukey tests. Letters (blue lowercase for bacterial system and brown capital for fungal system) indicate significant differences between means according to Tukey tests. Grey background indicates control treatment of bacterial and fungal systems.



855 **Figure 2 Effect of bacterial and fungal consumer - prey inoculations on bacterial community composition.** Discriminant function analysis of the bacterial PLFA markers in the (A) bacterial and (B) fungal systems. Overall differences in bacterial PLFA composition (8 markers) within the bacterial and fungal systems were analysed first by NMDS followed by MANOVA. Pairwise differences between means were further investigated using Mahalanobsis distances. For details of the results of the statistical tests see Table 2. RMB: remaining microbial background; coll. wash: collembolan wash. Ellipses encircle 75 % of
860 the data for visualisation purposes.



865 **Figure 3 Effect of bacterial and fungal consumer - prey inoculations on C dynamics.** (A) Microbial C use, indicated by the relative contribution of C from soil over litter in bacterial and fungal PLFA markers. Grey boxes indicate fungal PLFAs. (B) CO_2 production from soil microorganisms and animals; data are average emissions over 48 h after four and six weeks of incubation. Grey background indicates control treatment of both the bacterial and fungal system. (C) SOC concentrations. Differences between treatments were analysed separately for the bacterial and fungal systems using GLS models followed by
870 ANOVA and post-hoc Tukey tests. Letters (blue lowercase for bacterial system and brown capital for fungal system) indicate significant differences between means. In addition, in panel (A), for the four treatments of the fungal system, the differences in C origin in bacterial and fungal PLFAs were tested (pairwise comparisons).

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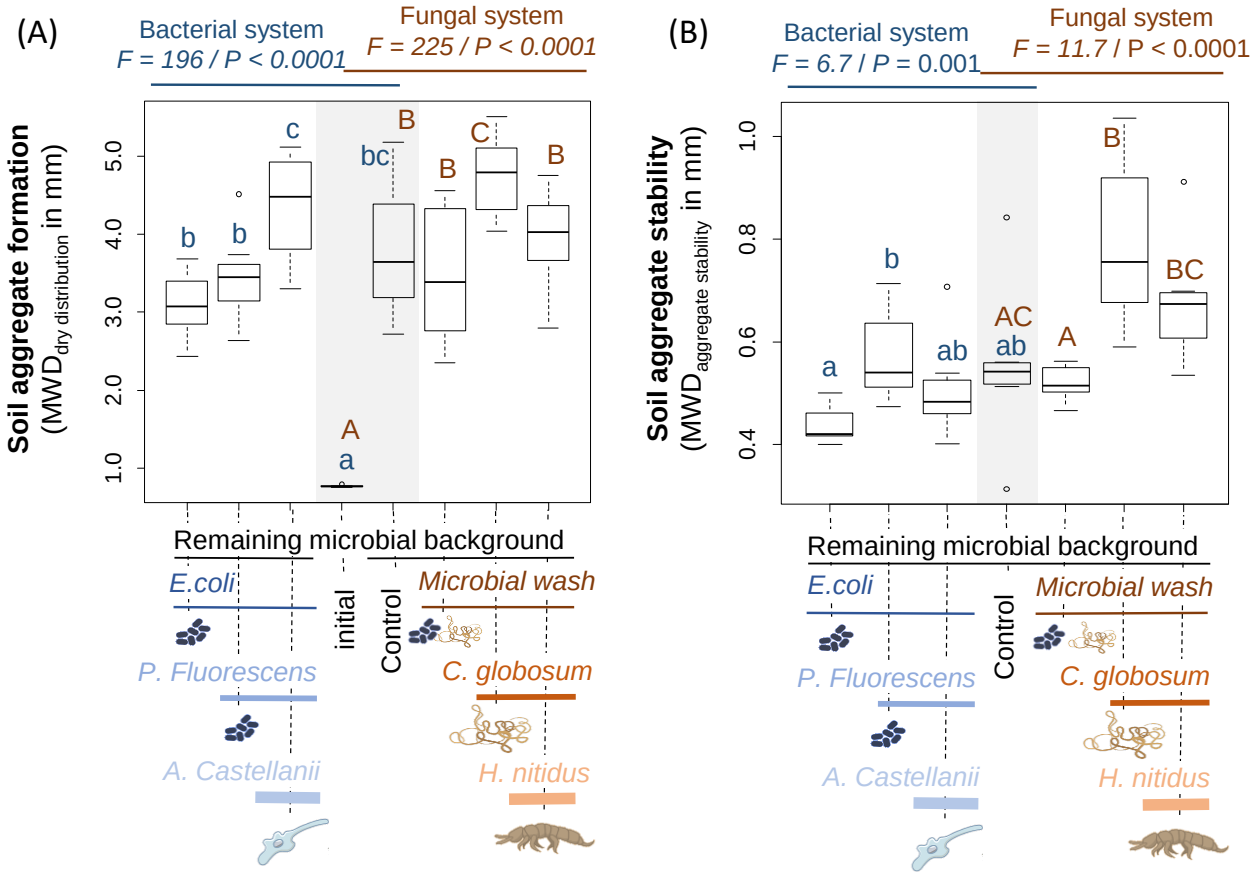

Figure 4 Effect of bacterial and fungal consumer - prey inoculations on (A) soil aggregate formation and (B) soil aggregate stability. Differences between treatments were analysed separately for the bacterial and fungal systems using GLS models followed by ANOVA and post-hoc Tukey tests. Letters (blue lowercase for bacterial system and brown capital for fungal system) indicate significant differences between means according to Tukey tests. Grey background indicates control treatments common to bacterial and fungal systems. ***$P < 0.001$; **$P < 0.01$.

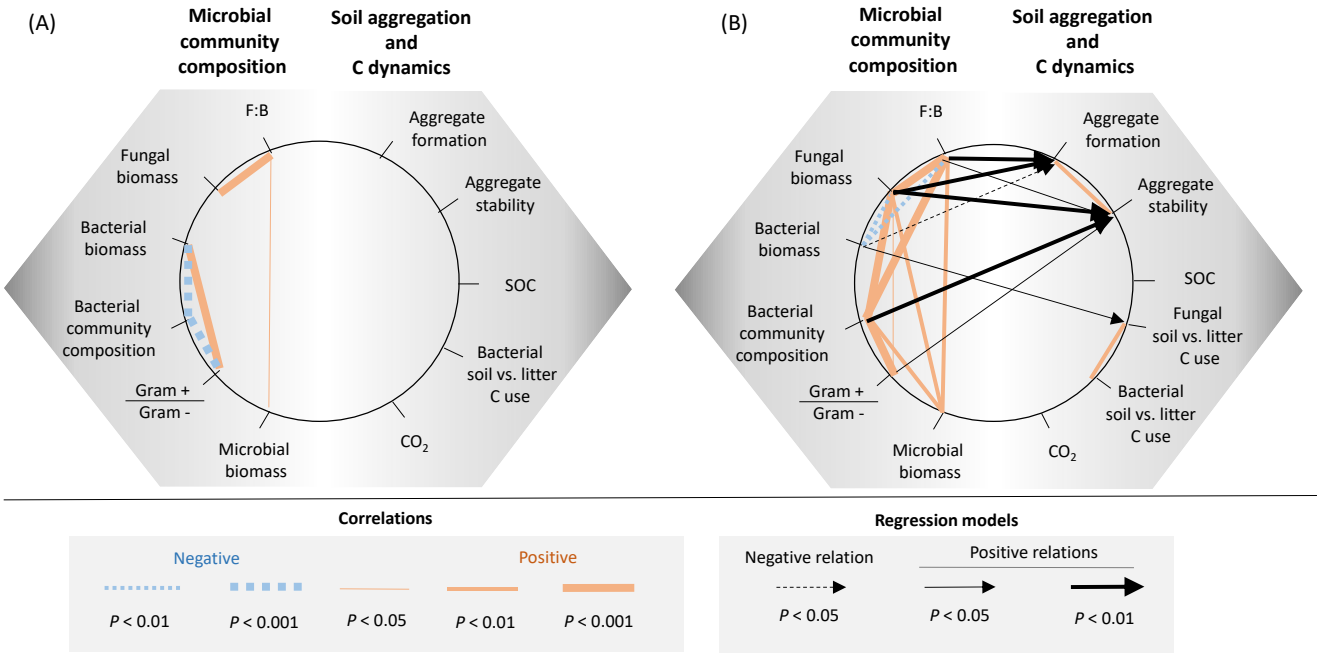

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Figure 5 Relationships between microbial community composition and soil aggregation and C dynamics in the (A) bacterial-based and (B) fungal-based systems. Coloured lines indicate signification correlations (Spearman) among descriptors of microbial community composition and soil functioning; for details of the statistical parameters see Table A1 and A2. Black arrows indicate significant relationships between soil functioning variables and descriptors of the microbial 890 community composition analysed by generalized linear models; for details of the statistical parameters see Table A3. Only significant relationships are displayed.

Table A1 Correlation matrix among descriptors of microbial community. Spearman's correlation coefficients (ρ) for the bacterial-based system (underlined letters) and the fungal-based system (bold letters).

	F:B	Fungal biomass	Bacterial biomass	Bacterial community composition	Gram + / Gram -	Microbial biomass
F:B	-	0.79 *P* < 0.0001	- 0.30 *P* = 0.09	0.31n.s *P* = 0.09	- 0.25 *P* = 0.16	0.39 *P* = 0.03
Fungal biomass	**0.99** ***P* < 0.0001**	-	0.24 *P* = 0.17	- 0.13 *P* = 0.5	0.16 *P* = 0.4	0.31 *P* = 0.08
Bacterial biomass	**- 0.64** ***P* = 0.0003**	**- 0.56** ***P* = 0.002**	-	- 0.69 *P* < 0.0001	0.58 *P* = 0.0007	0.032 *P* = 0.9
Bacterial community composition	**0.71** ***P* < 0.0001**	**0.75** ***P* < 0.0001**	**- 0.29** ***P* = 0.12**	-	- 0.74 P < 0.0001	0.29 *P* = 0.1
Gram + / Gram -	**0.29** ***P* = 0.13**	**0.39** ***P* = 0.04**	**0.31** ***P* = 0.09**	**0.71** ***P* < 0.0001**	-	0.06 *P* = 0.7
Microbial biomass	**0.56** ***P* = 0.002**	**0.54** ***P* = 0.003**	**- 0.32n.s** ***P* = 0.09**	**0.55** ***P* = 0.002**	**0.28** ***P* = 0.14**	-

Table A2 Correlation matrix among variables describing soil aggregation and overall C use. Spearman's correlation coefficients (ρ) for the bacterial-based system (underlined letters) and the fungal-based system (bold letters). NA indicates not applicable.

	Aggregate formation	Aggregate stability	Fungal soil C use	Bacterial soil C use	CO_2	SOC
Aggregate formation	-	0.16 *P* = 0.4	NA	0.07 *P* = 0.7	- 0.12 *P* = 0.5	- 0.07 *P* = 0.7
Aggregate stability	**0.46** ***P* = 0.009**	-		- 0.34n.s *P* = 0.06	0.21 *P* = 0.3	0.2 *P* = 0.2
Fungal soil C use	**- 0.38n.s** ***P* = 0.06**	**-0.15** ***P* = 0.5**	-		NA	NA
Bacterial soil C use	**- 0.09** ***P* = 0.6**	**- 0.07** ***P* = 0.7**	**0.59** ***P* = 0.003**	-	- 0.10 *P* = 0.6	- 0.03 *P* = 0.9
CO_2	**- 0.13** ***P* = 0.5**	**0.11** ***P* = 0.5**	**- 0.03** ***P* = 0.9**	**- 0.09** ***P* = 0.6**	-	- 0.11 *P* = 0.5
SOC	**0.13** ***P* = 0.4**	**- 0.05** ***P* = 0.8**	**- 0.03** ***P* = 0.8**	**- 0.07** ***P* = 0.7**	**0.04** ***P* = 0.8**	-

Table A3 Simple linear regressions between soil structure, C dynamics and descriptors of soil microbial community composition. Relationships were tested using simple generalised linear models (GLM); only significant relations are displayed.

		Statistical parameters			
	Explanatory variables	R^2	*P*	Slope	ΔAIC
Fungal system					
Aggregate formation					
	Fungal biomass	0.29	0.002	+	0
	F:B ratio	0.26	0.004	+	1.2
	Bacterial biomass	0.18	0.02	-	4.4
Aggregate stability					
	Bacterial community composition	0.28	0.002	+	0
	Fungal biomass	0.24	0.007	+	1.7
	F:B ratio	0.18	0.02	+	3.9
	Gram + / Gram -	0.16	0.03	+	4.6
Fungal soil vs. litter C use					
	Bacterial biomass	0.22	0.02	+	-

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Protists and collembolans alter microbial community composition, C dynamics and soil aggregation in simplified consumer - prey systems

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Abstract

Microbes play an essential role in soil functioning including~~, notably on~~ biogeochemical cyclinge~~s~~ and soil aggregate formation~~ion~~. Yet, a major challenge is to link microbes to higher trophic levels and assess consequences for soil functioning. Here, we aimed to assess how microbial consumers modify microbial community composition (PLFA markers), as well as C dynamics (microbial C use, SOC concentration~~s~~ and CO_2 emission~~s~~) and soil aggregation. We re-built two simplified soil consumer - prey systems: a bacterial-based system comprising amoebae (*Acanthamoeba castellanii*) feeding on a microbial community dominated by the free-living ~~bacteria~~ bacterium *Pseudomonas fluorescens*, and a fungal-based system comprising collembolans (*Heteromurus nitidus*) grazing on a microbial community dominated by the saprotrophic fungus *Chaetomium globosum*. The amoeba *A. castellanii* did not affect microbial biomass and composition, but enhanced the formation of soil aggregates, and tended to reduce their stability. Presumably, the dominance of *P. fluorescens*, able to produce antibiotic toxins in response to the attack by *A. castellanii* ~~attack~~, was the main cause of the unchanged microbial community composition, and~~.~~ ~~The~~ the release of bacterial extracellular compounds in reaction to predation, such as long-chained polymeric substances or proteases, ~~are thought to drive~~was responsible for the changes in soil aggregation as ~~a~~ side effect. In the fungal system, collembolans significantly modified microbial community composition~~, through~~ via consumptive and non-consumptive effects including the~~, notably via the~~ transport of microbes on the body surface. As expected, fungal biomass ~~was a main factor~~ promoted~~ing~~ soil aggregation~~,~~ and was ~~negatively modulated~~reduced in the presence of ~~the~~ *H. nitidus*. Remarkably, we also found an unexpected contribution of changes in bacterial community composition to soil aggregation. In both~~,~~ the bacterial and fungal systems, bacterial and fungal communities mainly consumed C from ~~the~~ soil ~~material~~ organic matter (rather than the litter added)~~in the microcosms~~. Increased fungal biomass was associated with an ~~improved~~ increased capture of C from added ~~chopped~~ litter and the presence of collembolans levelled-off this effect. N~~e~~either amoebae nor collembolans altered~~o changes in~~ SOC concentrations and CO_2 ~~emissions were observed~~production. Overall, ~~our work~~the reults demonstrated that

trophic interactions are important for achieving a mechanistic understanding of biological contributions to soil aggregation and ~~can~~ may occur without major changes in C dynamics, and with or without changes in the composition of the microbial community.

1. Introduction

Soil microbes are essential for soil functioning (Bardgett and Van der Putten, 2014). As major primary decomposers of ~~the~~ organic matter, soil microbial communities play a crucial role in soil biogeochemical cycles (Bradford et al., 2013; Graham et al., 2016; Glassman et al., 2018). Microbes ~~are~~ as well are key determinants of soil structure, notably soil aggregation (Lehmann et al., 2017). Soil aggregates are "soil-specific entities built from mineral and organic compounds with stronger bonds between building blocks than with neighbouring particles" (Yudina et al., 2019). Soil aggregate properties, in particular their stability, ~~are useful~~allow to predict large scale soil properties~~,~~ such as soil erosion (Barthes and Roose, 2002). The role of soil microbes on biogeochemical cycles and soil structure ha~~s~~ve been intensively studied in the past decades (Wieder et al., 2013; Lehmann et al., 2017; Malik et al., 2018). Yet, a major challenge is to link the soil microbial level to higher trophic levels (Grandy et al., 2016). To do so, it is required to ~~test~~ investigate how soil microbial grazers and predators impact the composition of the soil microbial community (Thakur and Geisen, 2019), and assess consequences for soil functioning.

Soil microbes form highly diverse and interconnected communities (Delgado-Baquerizo et al., 2016; de Vries et al., 2018; Waag et al., 2019), with bacteria and fungi as key players. ~~For~~~~Since~~In the last ~~a~~ decade, increasing effort has been undertaken to decipher ~~the~~ biotic interactions within microbial communities (Velicer and Vos, 2009; Schmidt et al., 2015; Deveau et al., 2018), and ultimately link~~ing~~ microbial diversity and connectivity to soil biogeochemical cycles (Waag et al., 2019). Bacteria and fungi interact in many ways, with numerous examples of competition and facilitation (Tiunov and Scheu, 2005; Velicer and Vos, 2009; Worrich et al., 2017; Braham et al., 2018), often resulting in complementary actions favouring biogeochemical cycles (Deveau et al., 2018; Waag et al., 2019). However, we know little about how the diversity and interactions within soil microbial communities drive soil structure. So far, general mechanisms underlying effects of bacteria and fungi on soil aggregation have been described using isolated microbial strains, generally pinpointing positive effects (Lehmann et al., 2017). Bacteria influence soil aggregation mainly through the production of extracellular polymeric substances (EPS), which adsorb onto mineral surfaces, increase the viscosity of the soil solution and enhance soil particle cohesion (Chenu, 1993; Sundhya and Ali, 2009; Liu et al., 2013). Filamentous fungi promote soil aggregation in various ways including (i) the enmeshment of soil particles (Degens et al., 1996; Tisdall et al., 1997; Baldock, 2002), (ii) the secretion of polymeric substances (Chenu, 1989; Caesar-TonThat and Cochran, 2000; Daynes et al., 2012), enhancing mineral particle cohesion similar~~ly~~ to bacterial EPS, and (iii) the release of a variety molecules, such as hydrophobins (Linder et al., 2005; Zheng et al., 2016) ~~or~~ and phenolic acids (Caesar-Tonthat and Cochran, 2000)~~, which reduce~~ reducing wettability and prevent~~ing~~ soil aggregates from collapsing when the ~~surrounding~~ soil is rewetted. However, effects vary between microbial strains. For example, good aggregators were

reported for species of the bacterial genera *Bacillus* and *Pseudomonas* (Caesar-TonThat et al., 2007; Caesar-TonThat et al., 2014), and of the fungal genera *Chaetomium*, *Mucor* and *Aspergillus* (Swaby, 1949). Enhanced aggregative ability ~~notably~~ was related to higher lipid and protein content ~~contents of lipids and proteins~~ in bacterial EPS (Liu et al., 2013), and to higher density of mycelial growth (Lehmann et al., 2020). Such developments with a microbial focus bring us closer to understanding how the diversity of microbes drive biogeochemical cycles and soil aggregation, but fail to connect how these processes are modified in the presence of higher trophic levels.

Soil microbial communities have long been thought to be mainly ~~driven~~ structured by abiotic filters (Griffiths et al., 2011) and plant inputs (bottom-up regulation~~s~~; Myers et al., 2001), whereas the importance of higher trophic levels is only yet seen as ~~a~~ major, but understudied factor (Thakur and Geisen, 2019) of~~, with~~ crucial importance for C cycling~~s~~ (Crowther et al., 2015; Filser et al., 2016; Grandy et al., 2016; Morriën et al., 2017). Several soil organisms can consume bacteria and fungi, including protists, nematodes and collembolans. These consumers often modify the biomass and the composition of the microbial community (Rønn et al., 2002; Djigal et al., 2004; Rosenberg et al., 2009; Perez et al., 2013; Jiang et al., 2017; Geisen et al., 2018; Coulibaly et al., 2019). Such modifications are thought to directly result from consumptive effects, i.e. selective feeding, or indirectly from changes in the competition between microbes due to the consumption of certain microbial strains (Thakur and Geisen, 2019). However, o~~O~~ther non-consumptive effects are ~~as well~~also at stake (Bradford et al., 2016) as indicated ~~, and are unequally supported~~ by experimental evidence~~s~~. For example, the release of N by protists and nematodes (Griffith and Bardgett, 1997), the excretion of urine or faeces by microarthropods (Cragg and Bardgett, 2001; Milcu et al., 2006), as well their necromass (Coleman et al., 2002) modify the quality of ~~the~~ organic matter processed by soil microorganisms, with expected effects on their biomass and composition. The comminution of plant debris (Lussenhopp, 1992) as well as the transport of microbes on the body of soil microarthropods (Griffith and Bardgett, 1997; Bardgett et al., 1998; Gormsen et al., 2004) ~~are~~ also are assumed to contribute to ~~the~~ non-consumptive effects of microarthropods on soil microbial communities. Further, consumers of soil microorganisms can modify microbial activity and physiology, such as the production of bacterial EPS (Matz and Kjellberg, 2005; Queck et al. 2006) ~~or~~ and antibiotic compounds (Jousset and Bonkowski, 2010) ~~in response to predation by protists~~. Variations in enzymatic activity of wood decomposing fungi in the presence of microbivores ~~as well have~~has also been reported ~~reported~~ (Crowther et al., 2015). Overall, the influence of trophic interactions on the structure and activity of soil microbial ~~community~~ communities ~~and activity~~ is an active research topic (Jiang et al., 2017; Thakur and Geisen, 2019; Lucas et al., 2020) ~~with already many experimental pieces of evidence accumulated~~. By contrast, the integration of higher trophic levels into soil aggregation studies lag far behind, with only one study investigating how collembolans modify the effect of fungi on soil aggregation (Siddiky et al., 2012) and no studies ~~reported~~ targeting at effects of protists or nematodes. However, given the importance of microbes ~~on~~ for soil aggregation, modifications in microbial community composition or activity due to microbial consumers are expected to play a significant role.

Here, we aimed at investigating how microbial consumers modify microbial community composition, and how this ~~further~~ impacts C dynamics and soil aggregation. We focused on two simplified consumer - prey systems: a bacterial- and a fungal-based system, representing the two main, and often interconnected, channels of C fluxes in soil (de Vries and Caruso, 2016). The bacterial-based system comprised the free-living amoeba~~e~~ *Acanthamoeba castellanii* feeding on a microbial community dominated by ~~the~~ bacteria (*Pseudomonas fluorescens* sensu lato), and the fungal-based system ~~comprised~~ comprising the ~~collembola~~collembolan species~~ns~~ *Heteromurus nitidus* grazing on a microbial community dominated by ~~the~~ saprotrophic fungi (~~us~~ *Chaetomium globosum*). We conducted a microcosm experiment ~~over~~ lasting for 6 weeks ~~duration~~ and assessed ~~resulting~~ changes in microbial community composition, C dynamics and soil aggregation.

2. Material and methods

2.1 Experimental design and microcosm incubation

We re-constructed simplified bacterial-based and fungal-based prey - consumers systems in soil microcosms. The soil was autoclaved (2 h at 121°C) to reduce and level-off initial microbial abundance and diversity, and re-inoculated with single common soil bacterial or saprotrophic fungal strains, i.e. *P. fluorescens* and *C. globosum*, to set up a bacterial- and a fungal-dominated system~~s~~, respectively. The resulting microbial community ~~was built out of~~established from the introduced species as well as species that survived ~~sterilization~~ autoclaving and those introduced together with the consumers, notably the microbes used to culture ~~the consumers~~the protists and collembolans in the laboratory (*Escherichia coli* and *Saccharomyces cerevisiae*, respectively). These microbial communities formed the prey of the consumers added. The consumers, i.e. the protist *A. castellanii* and collembolan *H. nitidus*, were added two weeks after the microbial inoculation to allow the microbial communities to establish. We accounted for the co-addition of microorganisms with the consumers to microcosms by adding *E. coli* and a microbial wash of the collembolans, respectively~~, to microcosms without adding the microbial consumers~~. In addition, we ~~had~~ set up a zero control in which only autoclaved tap water was added (for full experimental design see Table 1). Overall, our experimental design allowed to disentangle the effects of the addition of consumers on the microbial community due to (i) trophic interactions (consumptive~~s~~ and non-consumptive effects) from those related to (ii) the co-addition of microbes ~~carried on the body of~~with the consumers. Furthermore, we were able to assess how preceding steps of microbial community establishment influenced soil microbial community composition. We linked the changes in microbial community composition due to the preceding inoculation steps and the addition of consumers to C dynamics and soil aggregation.

In the bacterial~~l~~-based system, we first added *P. fluorescens* (2×10^9 cfu)~~,~~ and two weeks later~~,~~ *A. castellanii* (6×10^8 individuals) ~~was were added in~~ to half of the jars inoculated with *P. fluorescens*. In addition, the mucilage producing Gram-

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~~bacteria~~ bacterium *E. coli* (6×10^8 individuals; Danese et al., 2002), used to culture the amoebae, ~~was~~ ~~a~~s~~ere~~ added to the microcosms that did not receive~~d the~~ amoeba~~e~~ (for full experimental design ~~is provided in~~see Table 1). Bacteria and amoebae were added ~~in a~~as suspension ~~of~~ in autoclaved tap water ~~by addition of drops~~. In the fungal-based system, we first added the saprotrophic fungus~~i~~ *C. globosum* (4 cm^3 of colonized LB agar), and two weeks later the collembolan species *H. nitidus* (30 individuals per microcosm) ~~was added in~~to half of the jars inoculated with *C. globosum*. In addition, we added a microbial wash of collembolans (240 individuals) to the microcosms which did not receive~~d~~ the collembolans (for full experimental design ~~is provided in~~see Table 1). The microbial wash as well as *C. globosum* were added ~~in~~ as suspension in autoclaved tap water. *H. nitidus* was added using an entomological exhauster. The same amount~~s~~ of medium (autoclaved tap water with or within smashed LB agar) w~~as~~ere added to the respective control treatments.

The ~~bacteria~~ bacterium *P. fluorescens* and the fungus~~i~~ *C. globosum* were chosen as they are ~~usually~~ abundant in ~~natural~~ soils (Dubuis et al., 2007; Domsch et al., 1993) and ~~have a positive~~beneficially a ~~e~~ffect ~~on~~ soil aggregation (Swaby, 1949; Caesar-TonThat et al. 2014; Tisdall et al., 2012). The amoeba species *A. castellanii* was selected as ~~a~~ representative and ubiquitous soil-dwelling amoeba able to consume a broad range of microorganisms and for its ability to produce protease~~s~~ (Serrano-Luna et al., 2006; Weekers et al. 1995) able to perforate bacterial biofilms~~,~~ essential for soil aggregation. The amoeba species *A. castellanii* ~~is able~~was shown to feed on our model strain ~~of~~ *P. fluorescens* (Jousset et al. 2009), but prefers less toxic strains. We thus expected that the amoebae will not only feed on *P. fluorescens*, but also on the other bacterial strains of the system (remaining microbial background and *E. coli*). The collembolan species *H. nitidus* was chosen because of its abundance in European temperate soils (Hopkin, 1997) and ~~for its~~as it has been shown to preferentially feed on ~~appetence for~~ *C. globosum* (Pollierer et al., 2019); feeding on ~~. *H. nitidus* is show~~s~~ing a strong preference for~~ *C. globosum* ~~and~~ it is able to survive and reproduce ~~when fed on this saprotrophic fungus~~. *H. nitidus* ~~is~~ ~~as well~~also ~~able to~~ feed~~s~~ on bacteria including~~, notably~~ *P. fluorescens*, but cannot reproduce when fe~~e~~ding only this bacteria~~l species~~ (Pollierer et al. 2019). In our system, we thus expected that *H. nitidus* will mainly feed on *C. globosum*~~.~~, but ~~also~~ will also ingest~~s~~ ~~some~~ bacteria present in the microcosms.

The soil used in the ~~microcosms~~ experiment ~~was~~ comprised a mixture of sand (59.7%) and agricultural soil (39.8%), collected from a wheat agricultural experimental field of the University of Göttingen, managed under conventional tillage and located in the metropolitan area of Göttingen. The agricultural soil was crushed through a 1 mm sieve to destroy larger aggregates and then mixed with sand. The properties of the soil mixture were: 6.0% clay, 30.8% silt, 13.7% fine sand (63-200 µm), 41.4% medium sand (200-630 µm), 8.1% coarse sand (630-2000 µm), 4.5% $CaCO_3$ and 0.36% organic carbon (analyses were conducted by LUFA, Speyer, Germany, following the methods VDLUFA I, C2.2.1:2012 and DIN ISO 10694:1996-08). A total of 120 g dry weight of soil was added per microcosm.

To ~~test~~ analyse whether the use of soil C was influenced by consumer - prey interactions, we added a source of particulate organic matter, i.e. dried chopped litter (µm to mm pieces; 0.4%) to the soil mixture. The litter was composed of a mixture of

maize (85.3%) and wheat litter (14.7 %). ~~The dominance of litter originating from m~~Litter of maize~~,~~ as C4 plant~~,~~ was chosen to allow ~~trace~~ tracing ~~its~~ the incorporation of litter carbon into~~use by soil~~ microorganisms ~~by using their specific ^{13}C signal (C4 plants)~~. The organic carbon present in the agricultural soil ~~is~~ mainly derived from the residues of wheat ~~plant residues, a~~as C3 plant~~, and constitute the other main C source for microbes in the present experiment~~.

The microcosms consisted of glass jars (7.5 cm diameter and 10 cm high) containing the mixture of soil, sand and litter (120 g dry weight of soil mixture with litter) and were covered by non-sealed lids, allowing gas and water exchange, but limiting potential contamination in our semi-sterile experimental design. Microcosms were incubated in darkness in a climate chamber at 20°C for a total of 6 weeks (July – August 2017), with the first 2 weeks with microbial prey only. Soil water holding capacity was adjusted weekly to 60% of the maximum by adding autoclaved tap water ~~in the~~under sterile ~~hood~~conditions.

2.2 Microbial community composition ~~(phospholipids fatty acids)~~

Changes in soil microbial abundance and composition were quantified by phospholipid fatty acid (PLFA) analysis. Lipids were extracted from fresh soil equivalent to 3.5 g dry weight. The soil was frozen at -20ºC after the experiment until further use according to the protocol of Buyer at al. (2012). PLFAs were measured and identified as described in Pollierer et al. (2015) using a gas chromatograph (GC; Clarus 500 with Autosampler, Perkin Elmer, USA). The mass (nmol g^{-1} of dry soil) of all extracted and identified PLFAs was used as measure of microbial biomass. The PLFA 18:2ω6,9 was used as fungal biomarker and eight PLFAs were used as bacterial biomarkers: i15:0, a15:0, i16:0; i17:0 ($Gram^+$ bacteria), cy17:0, 18:1ω7 ($Gram^-$ bacteria), 16:1ω7 and 15:0 (general bacterial marker) (Frostegård & Bååth 1996; Contosta et al. 2015).

2.3 ~~Soil C dynamics: m~~Microbial C use, CO_2 emission and SOC concentration

The use of C originating from soil (organic C originally present in the wheat agricultural soil) vs. litter ~~origin~~ (added chopped litter mainly derived from maize; see below) by soil microbes was assessed by tracing the ^{13}C signal of ~~maize~~ the litter and ~~wheat tissues~~soil ~~(C4 and C3 plants, respectively)~~ in the microbial PLFA markers. In particular, we investigated the ^{13}C of the fungal PLFA marker and of the eight bacterial PLFA markers, which allowed us to trace the C source of fungal and bacterial communities. The ~~isotopic~~ $^{13}C/^{12}C$ ratios of the PLFAs ~~was~~ were measured using a trace gas chromatograph (GC; Thermo Finnigan, Bremen, Germany), equipped with a DB5-DB1 column ~~combination~~ (30 m and 15 m, both 0.25 µm ID, Agilent), and coupled via a GP interface to a Delta Plus mass spectrometer (Thermo Finnigan, Bremen, Germany). The temperature program was run according to the following steps: 1 min at 80˚C, an increase to 170˚C at a rate of 10˚C/min, an increase to 192°C at a rate of 0.7°C/min, 4 min at 192°C, an increase to 200°C at a rate of 0.7°C/min, an increase to 210°C at a rate of 1.5°C/min, a final increase to 300°C at a rate of 10°C/min, and a final step at 300°C during 10 min. Helium was used as carrier gas for injections (250°C). PLFAs were identified by comparison of their chromatographic retention times with those of standard mixtures composed of 37 different FAMEs (Fatty Acid Methyl Esters; Sigma Aldrich, St Louis, USA) ranging from

C11 to C24 and 26 BAMEs (Bacterial Fatty Acid Methyl Esters; Sigma Aldrich, St Louis, USA). Isotope ratios were expressed vs. Vienna Pee Dee Belemnite standard (V-PDB) as $\delta^{13}C$ [‰] = $(((^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard} - 1) * 1000$. To compare $^{13}C/^{12}C$ ratios of microbial PLFAs to the $^{13}C/^{12}C$ ratios of C sources (organic soil carbon and added litter), we also measured the $^{13}C/^{12}C$ ratios of the agricultural soil used in the microcosms and of the added litter using a Delta Plus mass spectrometer (Thermo Finnigan, Bremen, Germany). Aliquots of ca. 2 g of ~~pure~~ soil and litter samples were dried (70°C, 24 h), milled (45 s, frequency: 25/s; MM200, Retsch GmbH, Haan, Germany) and placed in a desiccator for 48 h. Aliquots of ca. 25 mg of ~~pure~~ soil and 0.7 mg of ~~pure~~ litter were analysed (eight replicates each). The difference between the average isotopic signature of the ~~pure~~ soil ($\delta^{13}C_{Soil}$ = -27.16 ± 0.06 ‰) and the ~~pure~~ litter ($\delta^{13}C_{litter}$ = -13.71 ± 0.06 ‰) covered an amplitude of 13.45 ‰ and set the full range of isotopic variation (100 %) used to ~~define~~ trace C origin. Using these values as endpoints ~~More precisely, the pure litter signature was set to 0 % of C from soil origin (and 100% of C from litter origin) and the pure soil signature to 100 % of C from soil origin. For each treatment and PLFA type (bacterial or fungal markers),~~ the proportion of C of soil origin in fungal and bacterial PLFA marker FAs (x) was calculated as follows: % C_{soil} ~~(of x)~~ = $[(\delta^{13}C_x - \delta^{13}C_{soil}) \times 100) / (\delta^{13}C_{litter} - \delta^{13}C_{soil})]$ with $\delta^{13}C_x$ the $\delta^{13}C$ signature of the respective marker FA. For bacterial markers, we used average $\delta^{13}C$ values weighted by the relative proportion of each bacterial marker considered. Using the mean $\delta^{13}C$ value of litter in this calculation assumes that carbon from maize and wheat litter in the litter mixture was used indiscriminately, which we assume to be justified considering the short period of time when predominantly labile litter compounds are used. Further, considering that the litter mixture comprised predominantly maize litter differential incorporation of carbon from wheat is likely to affect the calculations only little. As local hotspots of soil can show lower $\delta^{13}C$ signal than the average $\delta^{13}C$ signature of homogenised soil, the calculation may result in an estimated percentage of C of soil origin higher than 100 %.

~~Similarly, the added chopped litter shows a certain degree of heterogeneity in terms of δ13C signal as 14.7 % of this organic carbon is of wheat origin and the other dominating part is from maize origin. If microbes selectively feed on chopped wheat (or alternatively maize) litter, then a bias in the estimation of soil vs. added chopped litter C source is possible. However, such preferential feeding is unlikely to happen, notably because maize and wheat litter were homogenised before their addition to the soil mixture.~~

To monitor the activity of soil organisms (soil microbiota and soil animals), CO_2 production was measured twice during the incubation period by titration with HCl of 2 mL KOH placed in the jar for 48 h (Fig. 4), following the protocol of Macfadyen (1970). Finally, to estimate whether the inoculation treatments modified the soil organic carbon (SOC) content over the incubation period, ~~soil organic carbon~~SOC concentration of aliquots of soil from the treatments was measured after the end of the incubation period (Fig. 3). Aliquots (ca. 500 mg) of dried (105°C, 24 h) and milled (45 s, frequency: 25/s; MM200, Retsch GmbH, Haan, Germany) soils samples were placed at 600°C during 2 h to remove the organic carbon by combustion. Aliquots of 20 mg of milled soil (unburned and burned at 600°C) were used to measure their carbon content (Vario EL, Elementar, Hanau, Germany), which represented the inorganic and organic carbon, respectively. The soil organic carbon content was obtained by the difference in the concentrations between the total organic carbon and the inorganic carbon.

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2.5 Soil aggregate properties

Soil aggregate formation was assessed by soil dry sieving (6 helicoidal movements; 30 cm amplitude) of air-dried (ca. 22°C; 7 days) soil samples using the following sieves: 10 mm, 5 mm, 3 mm, 2 mm, 250 µm and 50 µm, resulting in seven diameter classes of aggregates. As soil was crushed through a 1 mm sieve during soil preparation for microcosm incubation, aggregates larger than 1 mm indicate enhanced cohesion acquired during incubation. The term "aggregate formation" is used to describe the amount and size of aggregates obtained by dry sieving after incubation. Soil aggregate stability was measured following ISO/FDIS (E) 10930 (2012) described in Le Bissonnais (1996) and Le Bissonnais and Arrouays (1997). Briefly, 8 g of dried (40°C, 24 h ~~at 40°C~~) soil aggregates (3 – 5 mm) were gently re-wetted by capillarity for 5 min on a buffer paper placed on a saturated sponge. Aggregates then were transferred into ethanol and aggregates > 50 µm were retrieved by sieving in ethanol. The aggregate fraction > 50 µm was dried at 40°C for 24 h and sieved using six sieves (2 mm, 1 mm, 500 µm, 200 µm, 100 µm, 50 µm), resulting in seven diameter classes of aggregates. The mean weight diameter (MWD) was calculated as the average diameter of aggregates weighted by the mass proportion of aggregates within each fraction. The MWD of the dry distribution of aggregates, indicating aggregate formation, is noted MWD_{dd}. The MWD obtained after dispersion of aggregate by gently rewetting, indicating aggregate stability, is noted MWD_{as}.

2.6 Data analyses

Differences between treatments in the concentration of microbial PLFA markers, microbial C use, CO_2 emissions, SOC concentrations and degree of soil aggregation were inspected using generalized least square (GLS) models, followed by ANOVA and post-hoc Tukey tests. Differences in variance between treatments were accounted for in the GLS models. The effects of treatments on bacterial PLFA composition (8 specific markers) were investigated using non-metric multidimensional scaling (NMDS), followed by discriminant function analysis (DFA). Overall differences in bacterial PLFA composition within the bacterial and fungal system first were analysed by MANOVA. Pairwise differences between treatments were further ~~tested~~ inspected using Mahalanobis distances. Changes in soil bacterial community composition werewas captured by the scores (Axis 1) of principal component analyses (PCA) of the eight bacterial PLFA markers for further analyses. Axis 1 of the PCA on bacterial markers of the bacterial and fungal system explained 39.3 % and 37.6 % of total variability, respectively. Pairwise correlations among (i) descriptors of soil microbial community and (ii) soil aggregation and C dynamics were tested using Spearman's correlation coefficients (ρ). Best explanatory variables of soil aggregation, microbial C use, SOC concentrations and CO_2 production were identified among the descriptors of the microbial community composition using simple linear generalized regression models (glm) and selected according to their R^2 and ΔAIC. Only significant models are displayed. All statistical analyses were run separately for the bacterial and fungal systems. Data provided in the text represent means ± standard deviation. All statistical analyses were conducted in R version 3.6.1 (R Development Core Team, 2008).

3. Results

3.1 Microbial community composition as affected by consumer - prey system

Overall, consumer - prey inoculation had only little influence on soil microbial biomass, but modified the composition of microbial communities (Fig. 1). In the bacterial system, the predator *A. castellanii* had did not significantly affect on total either microbial total biomass, fungal-to-bacterial (F : B) , PLFA ratio, or on bacterial community composition and the proportions of Gram$^+$ and Gram$^-$ bacteria (Figs. 1, 2, Table. 2). In the fungal system, the grazer *H. nitidus* significantly decreased fungal biomass, as well as the F : B ratio, but did not induce changes in microbial and bacterial biomass, or in the proportion of Gram$^+$ and Gram$^-$ bacteria (Fig. 1). Despite this lack of changes in specific bacterial markers, the addition of collembolans influenced the overall composition of the bacterial community (pairwise comparison, NMDS/DFA/Mahalanobsis distances; F = 4.8, P = 0.03< 0.05, Fig. 2, Table 2). Initial steps of microbial community establishment had as wellalso significantly influence affectedon the microbial community composition. In the bacterial system, treatments where *E. coli* and *P. fluorescens* were added showed significantly higher proportions of Gram$^-$ bacteria, and lower proportions of Gram$^+$ bacteria (Fig. 1 E-G). The oTotalverall bacterial biomass was lowest in both of these treatments (Fig. 1 C). In additionFurther, the addition of *P. fluorescens* significantly modified as well the bacterial community composition (pairwise comparison, NMDS/DFA/Mahalanobsis distances; F = 15.0;, P = 0.0002< 0.001, Fig. 2, Table 2), whereas the addition of *E. coli* had no significant effect (pairwise comparison, NMDS/DFA/Mahalanobsis distances; F = 5.2;, P => 0.05, Fig. 2, Table 2). In the fungal system, the inoculation of the microbial wash did not affect the fungal and the bacterial biomass, or the proportions of Gram$^+$ and Gram$^-$ bacteria (Fig. 1). However, the microbial wash significantly modified the overall bacterial community composition (pairwise comparison, NMDS/DFA/Mahalanobsis distances; F = 28.0;, P < 0.0001, Fig. 2, Table 2). The inoculation of *C. globosum* significantly increased fungal biomass and decreased bacterial biomass, which led to an overall increase in the F : B ratio (Fig. 1 B-D). Treatments where *C. globosum* was added showed a significantly lower proportion of Gram$^-$ bacteria and the addition of *C. globosum* the fungal strains significantly modified the overall bacterial community composition (NMDS/DFA/Mahalanobsis distances; F = 11.0;, P =< 0.0021). Overall, the bacterial community composition was significantly modified by every step of the establishment of the prey - consumer fungal-based system, as indicated by the significant differences between all pairs of treatments (Fig. 2, Table 2).

3.2 Microbial C use, CO_2 emissions and SOC concentrations as affected by consumer - prey system

Based on ^{13}C content in microbial PLFA markers, most of the bacterial (73 ± 14 % across all treatments) and fungal C (64 ± 22 %) originated from soil C (rather than litter C). In the bacterial systems these figures did not vary significantly among treatments (Fig. 3). The addition of the amoeba *A. castellanii* thus did not modify the use of C sources. In the fungal system, the origin of fungal and bacterial C differed in the fungal treatment. In microcosms with fungi only (F), 52 ± 11 % of fungal C originated from soil, whereas 72 ± 4.3 % of bacterial C was of soil origin, indicating that fungi captured more litter C

(48 ± 11 %) than bacteria (28 ± 4.3 %). These differences levelled off in the presence of collembolans (Fig. 3 A), indicating that the addition of *H. nitidus* modified the use of C sources by soil microbes. Despite these differences between fungi and bacteria in their ability to capture C resources, no significant differences were observed in terms of CO_2 emitted and SOC concentrations between treatments (Fig. 3 B, C).

3.3 Soil aggregation as affected by consumer - prey system

The 6 week incubation period in microcosms resulted in the formation of soil aggregates, regardless of the treatments considered (mean MWD_{dd} across all treatments = 3.84 ± 0.8 mm), compared to the initial soil conditions (MWD_{dd} = 0.77 ± 0.01 mm). The formed aggregates on average were unstable (mean MWD_{as} across all treatments = 0.58 ± 0.15 mm) and ranged from very unstable (min. MWD_{as}= 0.31 mm) to moderately stable (max. MWD_{as}= 1.04 mm), according to the classification of the international norm ISO/FDIS10930 (E) (2012). The protist and collembolan consumers had contrasting effects on soil aggregate formation. Adding *A. castellanii* significantly increased the formation of soil aggregates, whereas *H. nitidus* significantly decreased it (Fig. 4 A, B). Effects of consumers on soil aggregate stability were weaker and but more consistent in the bacterial and than in the fungal systems. Both *A. castellanii* and *H. nitidus* tended to reduce the stability of soil aggregates (Fig. 4 A, B). These effects were weak as no significant differences were observed by direct comparison of the treatments with *P. fluorescens* or *C. globosum* with and without their associated respective consumers. However, we observed that the significant increase in soil aggregate stability in response to the addition of *P. fluorescens* and *C. globosum* vanished when their associated consumers were added. Overall, this indicates that *A. castellanii* and *H. nitidus* reduce the positive effect of *P. fluorescens* and *C. globosum* on soil aggregate stability, although only weaklylittle. The initial inoculation of soil microorganisms also modified the formation and stability of soil aggregates. In particular, *P. fluorescens* increased the stability of the soil aggregates, but did not modify their formation (Fig. 4 A, B). *C. globosum* had a significant positive effect on both soil aggregate formation and stability (Fig. 4 A, B). Neither the addition of *E. coli* nor the microbial wash significantly modified soil aggregation.

3.4 Relationships between microbial community composition, C dynamics and soil aggregation

Descriptors of the microbial community composition were poorly related among each other in the bacterial system, whereas in the fungal system showed they were more correlatedions (Fig. 5, Table A1). In the bacterial system, the bacterial biomass positively correlated with the ratio of $Gram^+$ to $Gram^-$ bacteria ($\rho = 0.58$, $P <= 0.0071$). These two later variables also were significantly related to the microbial community composition (as indicated by PCA scores, $P < 0.001$). Despite overall low levels of fungal biomass in the bacterial system, the fungal biomass positively correlated with the F : B ratio ($\rho = 0.78$; $P < 0.0001$), which itself positively correlated with microbial biomass ($\rho = 0.39$, $P <= 0.035$). In the fungal system, the fungal biomass and the F : B ratio (themselves positively related, $\rho = 0.99$, $P < 0.001$) were positively correlated with the microbial biomass ($P <= 0.003$ and $P = 0.002$, respectively0.01). Fungal abundance (both fungal biomass and F : B ratio) also

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significantly correlated with the bacterial community composition (as indicated by PCA scores, $P < 0.0001$). The composition of the bacterial community was significantly related to the Gram$^+$ to Gram$^-$ bacteria ratio ($\rho = 0.71$; $P < 0.0001$), itself being related to fungal biomass ($\rho = 0.39$, $P = 0.04$), but not to the F : B ratio ($\rho = 0.29$; $P = 0.13$). Fungal biomass negatively correlated with bacterial biomass ($\rho = -0.64$, $P = 0.002$).

In both the bacterial and fungal systems, soil aggregation, microbial C use, CO_2 emissions and SOC concentrations were poorly correlated (Fig. 5, Table A2). In the bacterial system, no correlations were found between soil aggregate formation, soil aggregate stability, bacterial C use, CO_2 emissions and SOC concentrations (Fig. 5 A). In the fungal system, soil aggregate formation and stability were positively correlated ($\rho = 0.48$, $P = 0.009$). In addition, fungal C use positively correlated with bacterial C use ($\rho = 0.59$, $P = 0.03$), indicating that communities characterized by higher capture of litter C by fungi also presented higher incorporation of litter C into bacteria. SOC concentrations and CO_2 emissions were not related to each other, neither to microbial C use nor soil aggregation.

Variations in soil aggregation and C dynamics were not explained by any of the descriptors of the microbial community in the bacterial system (Fig. 5, Table A3). In the fungal system, some microbial community parameters were related to soil aggregation and overall C use (single generalized linear models). In particular, soil aggregate formation and stability both positively correlated to with fungal biomass ($P = 0.002$ and 0.007, respectively; Table A3) and the F : B ratio ($P = 0.01$ and 0.02, respectively; Table A3), which were strongly correlated variables (Table A2). Beside the dominant effect of fungal abundance (R^2 and AIC criteria), soil aggregate formation was negatively related to bacterial biomass ($R^2 = 0.18$, $P = 0.05$), but this . This relation was of lower strength less strong according to R^2 and AIC criteria (Fig. 5 B, Table A3). Concerning soil aggregate stability, the effects of fungal abundance, i.e. fungal biomass and F : B ratio, came second and third, respectively, behind following the effect of the bacterial community composition (R^2 and AIC criteria), which significantly correlated with soil aggregate stability ($R^2 = 0.28$, $P = 0.002$; Table A3). In addition, the ratio between Gram$^+$ and to Gram$^-$ bacteria ratio positively correlated with soil aggregate stability, but with less lower strongength ($R^2 = 0.16$, $P = 0.03$, higher AIC). Fungal C use positively correlated with bacterial biomass ($R^2 = 0.22$, $P = 0.02$) in the fungal system, indicating that fungi acquired more soil C when bacterial biomass was increased. The production of CO_2 and the SOC concentration was did not related correlate with to any microbial parameters in both the bacterial and fungal system.

4. Discussion

Our results showed that simplified trophic interactions modified microbial community composition and soil aggregation, but had low or no effects on did not or only little affect C dynamics. Overall, the effects were stronger in the fungali-based system than in , as compared as the bacterial-based system. In the latter, the inoculation of *P. fluorescens* as dominant bacterial strain in large drove the main changes in microbial community composition, whereas the addition of the amoeba predator *A.*

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~~C~~*castellanii* did not induced ~~detectable~~further changes, presumably because *P. ~~F~~fluorescens* is a less preferred and toxic strain for *A. castellanii*~~the amoeba~~. However, *A. ~~C~~castellanii* enhanced the formation of soil aggregates, presumably related to changes in the production of bacterial EPS~~,~~ in ~~reaction~~response to the attack by *A. castellanii*. In the fung~~i~~al-based system, ~~according~~conform to our expectations, the inoculation of *C. globosum* ~~induced an~~ increased ~~in~~ fungal biomass~~,~~ and the addition of the grazer *H. nitidus* reduced it. These variations in fungal biomass were positively related to changes in soil aggregation, ~~pointing~~suggesting a ~~negative~~~~detrimatal~~detrimental effect of ~~C~~collembolans on soil aggregation. ~~More s~~Surprisingly, the inoculation of *C. globosum* and *H. nitidus* resulted in significantly modified ~~the~~ bacterial biomass and composition, ~~which~~and this was ~~appeared to be also~~ related to changes in soil aggregation. Finally, in the bacterial- and fungal~~i~~-based systems, ~~the~~ soil ~~material~~organic matter was the dominant C source and inoculation steps only weakly modified the relative importance of soil vs. added chopped litter as microbial C source. ~~teably~~Notably, the inoculation treatments did not significantly affect~~changes in~~ SOC concentrations and CO_2 emissions ~~were reported~~, ~~pinpointing~~suggesting that despite trophic interactions significantly modify ~~can have fine effects on the~~ microbial community~~ies~~ and ~~the~~ soil aggregation ~~without major~~this may not change soil ~~changes in~~ C dynamics.

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4.1 Bacteria-based prey - consumer system

The composition of the microbial community was mainly driven by the addition of *P. fluorescens*, which dominated the system and is a less ~~-~~preferred toxic ~~strain~~ prey for *A. castellanii* (Jousset et al., 2009). As *P. fluorescens* is a Gram$^-$ bacterium, the increased proportion of Gram$^-$ bacteria in the system after its addition was expected ~~and is consistent with previous results~~. Interestingly, the addition of *P. fluorescens* also decreased the proportion of Gram$^+$ bacteria as well as ~~the~~ overall bacterial biomass. Thus, *P. fluorescens* ~~thus~~ presumably constrained the growth of the remaining microbial background community present in the system, notably by outcompeting Gram$^+$ bacteria (Powers et al., 2015).

The addition of the amoeba *A. castellanii* overall weakly affected bacterial abundance and community composition. This lack of effect contrasts ~~with~~ previous work report~~detect~~ing a ~~sharp~~ strong decrease in bacterial biomass~~,~~ as well as significant and rapid changes in bacterial community composition in presence of *A. castellanii* (Jousset et al., 2009; Rosenberg et al., 2009). One possible explanation for this discrepancy is the dominance of *P. fluorescens* and its ability to produce ~~antibiotic~~ toxins in response to grazing by *A. castellanii* (Jousset and Bonkowski, 2010). In fact, *P. fluorescens* usually is not a preferred prey for *A. castellanii* (Jousset et al., 2009). In our system, the amoebae thus presumably only moderately fe~~e~~d on *P. fluorescens*, and exerted stronger pressure on the other ~~and~~ less abundant bacterial strains, namely *E. coli* and the bacteria from the remaining microbial background. As a consequence, there may have been changes in the composition of non-toxic bacterial strains, and in the proportions of Gram$^-$ and Gram$^+$ bacteria, the latter usually being less preferred by protozoa due to their protective cell wall (Ronn et al., 2002, Murase et al., 2006). However, protected from protozoan predation the dominance of *P. fluorescens*~~,~~ ~~which presumably remained weakly affected by protozoan predation because of toxin production,~~ masked these changes. In addition, due to prefer~~ential~~~~red~~ feeding on non-toxic strains, one could have expected beneficial effects ~~on the growth of~~of

amoebae on *P. fluorescens*, but no increase in Gram⁻ bacteria occurred in treatments with *A. castellanii*. This lack of change may have resulted from several concomitant factors. First, *P. fluorescens* was ~~already~~ dominating the bacterial community, leading to little release in the competitive pressure ~~caused~~ due to~~by~~ preying on the less abundant non-toxic ~~minority strains~~bacteria. Further~~more~~, low abundance of non-toxic bacterial strains in our system resulted in low nutrient release due to grazing by protists on these bacteria (Jousset et al. 2008). The potential benefit for the growth of *P. fluorescens* thus presumably was minor. Moreover, although *P. fluorescens* is a non-preferred prey it~~, but~~ nonetheless is consumed by *A. castellanii* (Jousset and Bonkowski, 2010), and this may have counteracted positive effects on its growth. Finally, the activation of toxin production by *P. fluorescens* is costly (Zha et al., 2006), and the production of such defence molecules usually is associated with reduced investment in bacterial growth (Malik et al., 2020). A more detailed investigation of bacterial community composition using molecular tools may have allowed deeper insight into these processes and should be employed in future studies.

Soil aggregation, but not bacterial C use, SOC concentrations and CO_2 production, was modified by the inoculation of the consumer - prey system. The bacterial community incorporated most of its C from soil (73.4 ± 14.0 %) and this remained unchanged regardless of the inoculation treatment. ~~The higher scattering~~More homogeneous distribution of soil organic matter, already processed and embedded into the soil matrix, compared to the added chopped litter, composed of relatively large pieces (µm to mm size) of non-processed plant debris, may explain this high incorporation (Malik et al., 2016; Tecon and Or, 2017). The unchanged proportion of C sources by bacteria when specific bacterial strains or the amoeba predator were added~~,~~ is consistent with the lack of effect of these inoculation steps on ~~the~~ overall microbial biomass. The unchanged levels in CO_2 emission and SOC concentrations as well were in line with the constant microbial biomass. What was more surprising is that despite ~~no changes in~~ microbial biomass and composition~~, or in~~as well as C dynamics remained unchanged, we observed modifications in soil aggregation after the amoeba predator was added. To the best of our knowledge, this is the first experimental evidence that protists affect soil aggregation, and that significant effects of amoebae can occur without modifying the biomass and the composition of the microbial community. Possible explanations are expected changes in microbial activity. In our case, *P. fluorescens* was dominant and presumably weakly affected by *A. castellanii* in terms of biomass, but both may have ~~changes~~ changed ~~in its~~their activity ~~should definitively have occurred~~. In particular, *P. fluoresencens* responds to predation by *A. castellanii* through enhanced production of phenolic compounds with antibiotic properties (Jousset and Bonkowski, 2010)~~, and upregulate the production of proteases enabling the amoebae to perforate bacterial biofilms (Serrano-Luna et al., 2006).~~ Enhanced mucilage production, with higher production of polysaccharides, ~~is~~ as well is a common strategy used by bacteria in response to predation by protists, leading to higher bacterial survival and growth (Matz and Kjellberg, 2005; Queck et al. 2006). Further, *A. castellanii* may have upregulated the production of proteases enabling the amoebae to perforate bacterial biofilms (Serrano-Luna et al., 2006). All these compounds released by the amoeba predator ~~or~~ and its bacterial prey have a significant potential to induce changes in soil aggregation as side effect. Polysaccharides (Chenu, 1989; Chenu, 1993), proteins (Rillig et al., 2004; Liu et al., 2013; Erktan et al., 2017) and phenolic compounds (Yoshikawa et al., 2018) are all known for their important role in soil aggregation. It is plausible that an enhanced production of mucilage, notably

polysaccharides, by *P. fluorescens* may be the underlying cause of the formation of larger soil aggregates after the addition of *A. castellanii*. Furthermore, the release of proteases by *A. castellanii* may have decreased the wettability of the mucilage, and thereby have been responsible for the lack of associated significant increase in the stability of aggregates. Although our focus was not to quantify changes in extracellular compounds occurring in amoeba - bacteria interactions, our results suggest that further investigations in this direction may be promising, and could explain the effects of protists on soil aggregation. Initial steps of the inoculation of the prey - consumer system also influenced soil aggregation. In absence of the amoeba predator, the bacterial communities, activated by wet and dry cycles (Cosentino et al., 2006), promoted the formation of soil aggregates. This effect occurred regardless of the bacterial strains inoculated, suggesting a similar ability of these bacterial strains to increase soil particle cohesion. By contrast, the inoculation of *P. fluorescens* significantly stabilised ~~the~~ soil aggregates, suggesting specific hydrophobic properties of its EPS.

Overall, the effects of the amoeba *A. castellanii* on the microbial community were limited, and ~~did not~~neither induced changes in microbial biomass and composition~~,~~ nor in C dynamics. ~~Despite of that~~However, despite this lack of effects the addition of the amoeba predator ~~effects~~ significantly affected~~on~~ soil aggregation ~~were found after the addition of the amoeba predator~~. Such changes in soil aggregation ~~independently~~irrespective of changes in C dynamics and microbial composition ~~,~~ suggest that protists preying on bacterial communities can significantly affect soil structure, without significantly affecting microbial growth and C cycling~~es~~. Presumably, changes in the activity of microbiota, notably the release of extracellular compounds in ~~reaction~~ response to predation by protists, are responsible for changes in soil aggregation as side effects.

4.2 Fungi-based prey ~~-~~ consumer system

The microbial community was influenced by every inoculation step in the fungal~~i~~-based system including~~, notably~~ by the addition of the collembolan grazers, and showed strong relations between the fungal and bacterial communities. As expected, fungal biomass was highest in the treatment with *C. globosum*, indicating that our inoculation was successful and triggered fungal development, which drove an overall increase in microbial biomass. Remarkably, the increase in fungal biomass was associated with a decrease in bacterial biomass~~,~~ as well as in Gram$^-$ bacteria, indicating competition between fungi and bacteria~~ng effects~~ as is ~~, reflecting common interactions~~common in the top soil (Braham et al., 2018). Adding collembolans reduced fungal biomass, presumably ~~because~~ due to~~of~~ the consumption of fungal hyphae by *H. nitidus*, confirming that *C. globosum* is consumed by *H. nitidus* (Pollierer et al., 2019). In addition, as collembolans also are feeding on litter (Potapov et al. 2016), competition for litter resources between fungi and collembolans may have contributed to the reduced fungal biomass. ~~Curiously~~Unexpectedly, ~~, the~~ bacterial biomass did not benefit from the decrease in fungal biomass ~~in response~~due to ~~the grazer~~the addition of *H. nitidus*. ~~Two reasons can explain this~~This might have been due to~~,~~ (i) the competitive pressure exerted by ~~the~~ fungi remained high in the presence of *H. nitidus*, and/or (ii) the potential positive effects of ~~a~~ reduced competition ~~with *C. globosum*~~ were compensated by *H. nitidus* also feeding ~~as well~~ on bacteria (Pollierer et al., 2019), even though probably at ~~a~~ low level. ~~Remarkably~~In fact, the composition of the bacterial community was significantly modified by collembolans.

Presumably, these changes were due to selective feeding of *H. nitidus* on certain bacterial strains, and/or through modifications in competitive interactions between certain bacterial strains and *C. globosum* ~~fungi~~ (Lussenhopp, 1992; Coulibaly et al., 2019; Thakhur and Geisen, 2019). In addition, other non-consumptive effects may have ~~been~~ occurred. For example, ~~the~~ comminution of the added litter by collembolans ~~pieces~~ (Coleman et al., 2002) may have contributed to changes in bacterial community composition (Scheu et al., 2005). ~~The~~ Further, the release of specific organic compounds by living or dead collembolans, such as urine, faeces (Lussenhop, 1992) or chitin (Lucas et al., 2019), ~~as well~~also may have modified the composition of the bacterial community. Not~~abiceab~~ly, the addition of the collembolan wash also significantly modified the bacterial community composition. Although it has long been assumed ~~for long~~ that microorganisms from the body surface of microarthropods may affect soil microbiota (Lussenhopp, 1992; Scheu et al., 2005), our results provide experimental evidence for it through the separate addition of a microbial wash. This effect highlights the importance of processes of community coalescence (Rillig et al., 2015) caused by the carriage of ~~a~~ microorganisms~~bial community~~ on the body of the consumers~~, which encounter the microbial community already present in the soil~~. Far from anecdotic, these effects were as strong as those ~~observed~~ when the soil animals themselves were added, suggesting that future studies should integrate such non-consumptive effects as main driver of the role of microarthropod grazers on soil microbial community composition.

Modifications in the composition of the microbial community were associated ~~with~~ by changes in microbial C use and soil aggregation, but not by changes in SOC concentrations and CO_2 emissions. More C of litter origin was captured by fungi rather than bacteria when fungal biomass was high~~est~~. ~~Higher~~ Superior ability of fungi than bacteria in ~~to~~ capture~~ing~~ litter C ~~was~~ has been ~~already~~ observed previously in microcosm ~~conditions~~ experiments (Malik et al., 2016), and is consistent with the network structure of fungal mycelia~~,~~ able to bridge air-filled pores (Otten et al. 2001)~~,~~, and thus able to reach distant resources~~,~~ such as pieces of litter widely distributed in the soil matrix. In our study, the increased use of litter C by fungi was ~~linked to a greater~~associated by an increased use of litter C by bacteria. This positive correlation suggests that fungi facilitated the access of litter C to bacteria, confirming previous similar observations (Purahong et al., 2016; Gorka et al., 2019). This positive effect may ~~be caused by~~have been due to the transfer of nutrients by fungal hyphae (Worrich et al., 2017) or ~~the~~ increased mobility of ~~the~~ bacteria along ~~the~~ fungal ~~highway~~ hyphae (Simon et al. 2015; Worrich et al., 2016). Effects, however, were minor ~~as no~~and did not affect ~~overall changes in~~ the bacterial C source, ~~were~~was observed between treatments and~~which~~ remained to be based mainly on soil C ~~remained the main source of carbon for bacterial communities~~. Interestingly, all these changes in microbial biomass, composition and C use occurred without impacting the overall production of CO_2~~, or~~ and ~~the~~ SOC concentration. Potentially, concomitant opposite effects of increased microbial biomass and decreased microbial activity have been responsible for the overall constant levels of soil respiration. Such compensatory effects have ~~already~~ been proposed previously to be responsible for ~~to explain~~ the absence of effects of higher trophic level consumers on soil respiration (Mikola and Setälä, 1998; Lucas et al., 2020).

The positive effect of *C. globosum* on soil aggregation is consistent with previous studies highlighting ~~a~~ positive effects of fungi, in particular saprotrophic ones, on soil aggregation (Caesar-TonThat, 2000; Daynes et al., 2012; Lehmann et al., 2017), especially when fungi dominate the microbial community (McMalla et al., 1958). Collembolans reduced the positive effect of *C. globosum* on soil aggregation. As ~~we expected~~hypothesized, the reduction in fungal biomass by collembolans ~~was a major factor explaining~~presumably was responsible for the negative effect of *H. nitidus* on soil aggregation in our system. ~~Perhaps what was m~~More intriguing ~~about our findings was that~~ changes in bacterial community composition~~,~~ related to ~~several~~ inoculation steps~~,~~ also contributed to ~~explaining~~ variations in soil aggregate stability. Previous studies reported positive effects of ~~fungi~~ interactions between~~ng~~ fungi ~~with~~ and bacteria on soil aggregation (Aspiras et al., 1971) as well as differential ~~aggregating ability~~effects of Gram$^+$ and Gram$^-$ bacteria ~~taken in isolation~~ (Mu'minah et al., 2015). ~~Here, we go further by pinpointing~~Our results further suggest that changes in the bacterial community composition contribute to a similar ~~level~~ extent as fungal biomass to ~~explain~~ changes in soil aggregate stability. Our design does not allow to disentangle the ~~specific~~ individual effects of fungal biomass and bacterial community composition, but we suggest that a combination of both ~~is~~ was responsible for the observed overall effects on soil aggregate stability. Altogether, we showed that collembolans modified the microbial community composition, presumably through ~~several~~ both consumptive and non-consumptive effects, which impacted soil aggregation.

5. Conclusions

We demonstrated that simplified consumer - prey interactions influence soil microbial community composition, microbial C use, soil aggregation, but not CO_2 emissions~~, nor~~ and SOC concentrations. Remarkably, effects on soil aggregation were not related to SOC or~~, neither~~ CO_2 emissions, suggesting that ~~fine~~ subtle but significant effects related to trophic interactions can occur at constant levels of SOC and microbial respiration. In the bacterial-based system, changes in soil aggregation occurred without any modification in microbial biomass and community composition. This lack of change presumably resulted from the dominance of *P. fluorescens*, a toxic and non-preferred prey for *A. castellanii*. We conjecture that changes in bacterial physiology, such as increased EPS production and/or the release of secondary metabolites in response to predation, is the main cause of the formation of larger aggregates. In the fungal-based system, the collembolan species *H. nitidus* significantly modified the microbial community~~, notably through a~~via ~~decreased~~ decreasing the biomass of *C. globosum* and ~~significant~~ chang~~es~~ing ~~in~~ bacterial community composition. Not~~ice~~ably, we found that the effects of collembolans on the soil microbial community did not only result from consumptive effects, ~~and pinpointed~~suggesting that the transport of soil microorganisms~~bes~~ on the body surface of collembolans is of major importance ~~to decipher~~for the effect of higher trophic levels on ~~the~~ soil microbial communities. As expected, fungal biomass was a main factor promoting soil aggregation, and was negatively modulated in the presence of the fungal grazer *H. nitidus*. Remarkably, we also found an unexpected contribution of changes in bacterial community composition on soil aggregation. We conclude that the effects of consumer - prey interactions on soil aggregation can be either positive or negative and were in our case stronger ~~on~~ on the formation of soil aggregates than on their stabilisation ~~formation~~. Our results highlight that non-trivial changes in microbial community

composition and/or activity caused by microbial consumers impact soil aggregation as side effects. Integration of more complex trophic interactions are ~~further~~ needed to assess how the mechanisms highlighted in our simplified systems are embedded into more complex processes in natural systems.

6. Data availability

In the case of acceptance of the manuscript, the data supporting the results will be archived in a public repository (Dryad or Zenodo) and the data DOI will be included at the end of the article.

7. Author contribution

AE and SS designed the study. MR, AJ and AC provided advise on the experimental design. AE conducted the microcosms experiment. AJ provided the culture of *Acanthamoebae castellanii*. AE analysed the PLFAs data. AE ran the statistical analyses and wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

8. Competing interests

The authors declare that they have no conflict of interest.

9. Acknowledgements

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Tables

Table 1: Experimental design
Details on the experimental design. X indicate which soil organisms have been added to the systems.

		Bacterial based system			Fungal based system			
Treatment		**Prey**	**Prey and consumer**	**Consumers associated microbiota**	**Prey**	**Prey and Consumer**	**Consumers associated microbiota**	**Common zero control**
Specific bacterial and fungal strains	*Pseudomonas fluorescens*	X	X					
	Chaetomium globosum				X	X		
Consumers	*Acanthamoeba castellanii*		X					
	Heteromorus nitidus					X		
Consumers associated microbiota	*Escherichia coli*	X	(X) Directly added by predator inoculation	X				
	Microbial wash of Collembolan				X	(X) Directly added by grazer inoculation	X	

Table 2 Effect of bacterial and fungal consumer - prey inoculations on bacterial community composition. Differences in the composition of bacterial PLFAs between treatments ~~in terms of bacterial PLFAs composition~~ were analysed using non-metric multidimensional ~~analysis~~ scaling (NMDS), followed by discriminant function analysis (DFA). Overall differences in terms of bacterial PLFA~~s~~ composition (8 markers) within the bacterial and fungal systems were first ~~tested~~ analysed by ~~Manova~~ MANOVA~~(F; *P*)~~. Pairwise differences between treatments were further investigated using Mahalanobsis distances ~~(F; *P*)~~. ~~We underlined the differences in terms of i~~Inoculation ~~within each pair of~~ treatments are underlined. ~~Abbreviations are:~~ RMB: remaining microbial background; coll. wash: collembolan wash.

Pair of treatments (a // b)	Statistical parameters		
	F-value	*P-value*	Mahalanobsis distance
Bacterial system			
Overall differences (~~Manova~~MANOVA)	14	***<0.0001	-
RMB + *E. coli* + *P. fluo* | RMB + *E. coli* + *P. fluo* + *A. castellanii*	2.1	~~n.s~~0.21	0.9
RMB + *E. coli* + *P. fluo* | RMB + *E. coli*	15	***0.0002	3.2
RMB + *E. coli* + *P. fluo* | RMB	172	<0.0001****	4.9
RMB + *E. coli* | RMB + *E. coli* + *P. fluo* + *A. castellanii*	17	***0.0001	4.0
RMB | RMB + *E. coli* + *P. fluo* + *A. castellanii*	115	***<0.0001	5.1
RMB | RMB + *E. coli*	5.2	~~n.s~~0.04	2.7
Fungal system			
Overall differences (~~Manova~~MANOVA)	16	***<0.0001	-
RMB + coll. wash + *C. globosum* | RMB + coll. wash + *C. globosum* + *H. nitidus*	4.8	*0.03	3.2
RMB + coll. wash + *C. globosum* | RMB + coll. Wash	11	**0.002	3.5
RMB + coll. wash + *C. globosum* | RMB	42	***<0.0001	6.4
RMB + coll. wash | RMB + coll. wash + *C. globosum* + *H. nitidus*	13	***0.0008	3.7
RMB | RMB + coll. wash + *C. globosum* + *H. nitidus*	49	***<0.0001	5.4
RMB | RMB + coll. wash	28	***<0.0001	4.0

~~***P<0.001; *P<0.05; n.s: P>0.05~~

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Figures

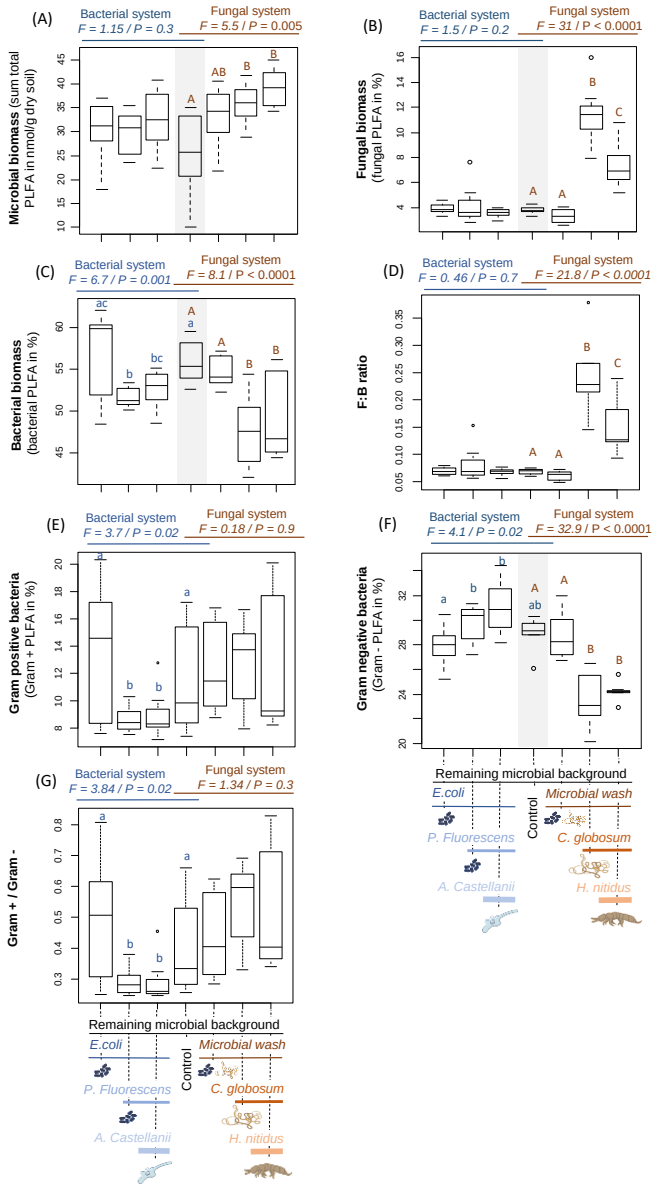
(A)
Bacterial system
F = 1.15 / P = 0.3
Fungal system
F = 5.5 / P = 0.005
Microbial biomass (sum total PLFA in nmol/g dry soil)
(B)
Bacterial system
F = 1.5 / P = 0.2
Fungal system
F = 31 / P < 0.0001
Fungal biomass (fungal PLFA in %)
(C)
Bacterial system
F = 6.7 / P = 0.001
Fungal system
F = 8.1 / P < 0.0001
Bacterial biomass (bacterial PLFA in %)
(D)
Bacterial system
F = 0. 46 / P = 0.7
Fungal system
F = 21.8 / P < 0.0001
F:B ratio
(E)
Bacterial system
F = 3.7 / P = 0.02
Fungal system
F = 0.18 / P = 0.9
Gram positive bacteria (Gram + PLFA in %)
(F)
Bacterial system
F = 4.1 / P = 0.02
Fungal system
F = 32.9 / P < 0.0001
Gram negative bacteria (Gram - PLFA in %)
(G)
Bacterial system
F = 3.84 / P = 0.02
Fungal system
F = 1.34 / P = 0.3
Gram + / Gram -
Remaining microbial background
E.coli
P. Fluorescens
A. Castellanii
Control
Microbial wash
C. globosum
H. nitidus

Figure 1 Effect of bacterial and fungal consumer - prey inoculations on microbial biomass and composition. (A) Microbial biomass, (B) fungal biomass~~, and~~ (C) bacterial biomass ~~indicated by the sum of total PLFAs and proportions of fungal and bacterial PLFA markers, respectively;~~, (D) ~~Fungal~~ fungal : ~~Bacterial~~ bacterial PLFA ratio (F : B~~);~~), (E) proportion~~s~~ of Gram$^+$ bacteria and (F) Gram$^-$ bacteria~~ ,indicated by the proportion of their associated PLFA markers;~~ and (G) ratio between Gram$^+$ and Gram$^-$ bacteria. Differences between treatments were ~~tested~~ analysed separately ~~within~~ for the bacterial and fungal systems using GLS models~~,~~ followed by ANOVA and post-hoc Tukey tests. Letters (blue lowercase for bacterial system and brown capital for fungal system) indicate significant differences between means according to Tukey tests. Grey background indicates control treatment of bacterial and fungal systems.

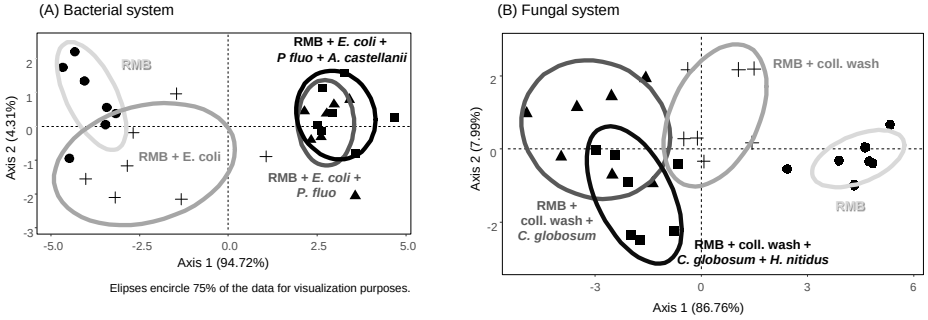

Figure 2 Effect of bacterial and fungal consumer - prey inoculations on bacterial community composition. Discriminant function analysis of the bacterial PLFA markers in the (A) bacterial and (B) fungal systems. Overall differences in bacterial PLFA composition (8 markers) within the bacterial and fungal systems were ~~tested~~ analysed first by NMDS followed by MANOVA. Pairwise differences between means were further investigated using Mahalanobsis distances. ~~Details~~ For details of the results of the~~se~~ statistical tests ~~are provided in~~see Table 2. RMB: remaining microbial background; coll. wash: collembolan wash. Ellipses encircle 75 % of the data for visualisation purposes.

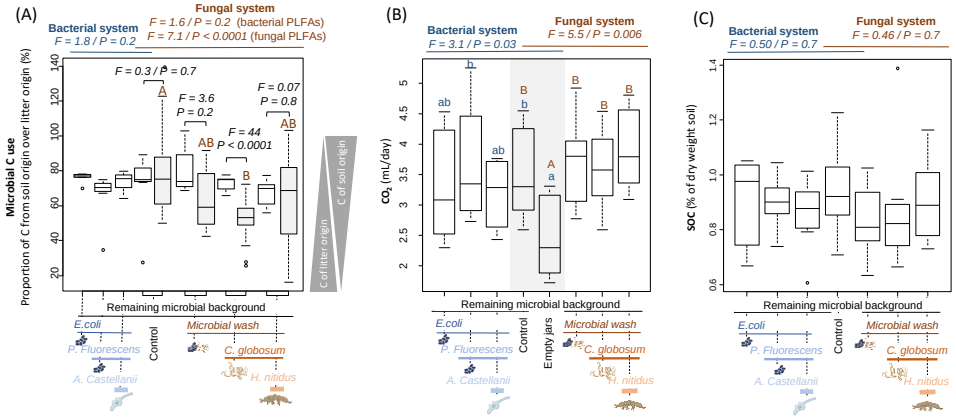

Figure 3 Effect of bacterial and fungal consumer - prey inoculations on C dynamics. (A) Microbial C use, indicated by the relative contribution of C from soil over litter ~~origin~~ in bacterial and fungal PLFA markers. Grey boxes indicate fungal PLFAs. (B) CO_2 production from soil micro<u>organisms</u>~~bes~~ and animals; data are average emissions over 48 h after four and six weeks of incubation. Grey background indicates control treatment of both the bacterial and fungal system. (C) SOC concentrations. Differences between treatments were ~~tested~~ <u>analysed</u> separately ~~within~~ <u>for the</u> bacterial and fungal systems using GLS models followed by ANOVA and post-hoc Tukey tests. Letters (blue lowercase for bacterial system and brown capital for fungal system) indicate significant differences between means. In addition, in panel (A), for the four treatments of the fungal system, the differences in C origin in bacterial and fungal PLFAs were ~~similarly~~ tested (pairwise comparisons).

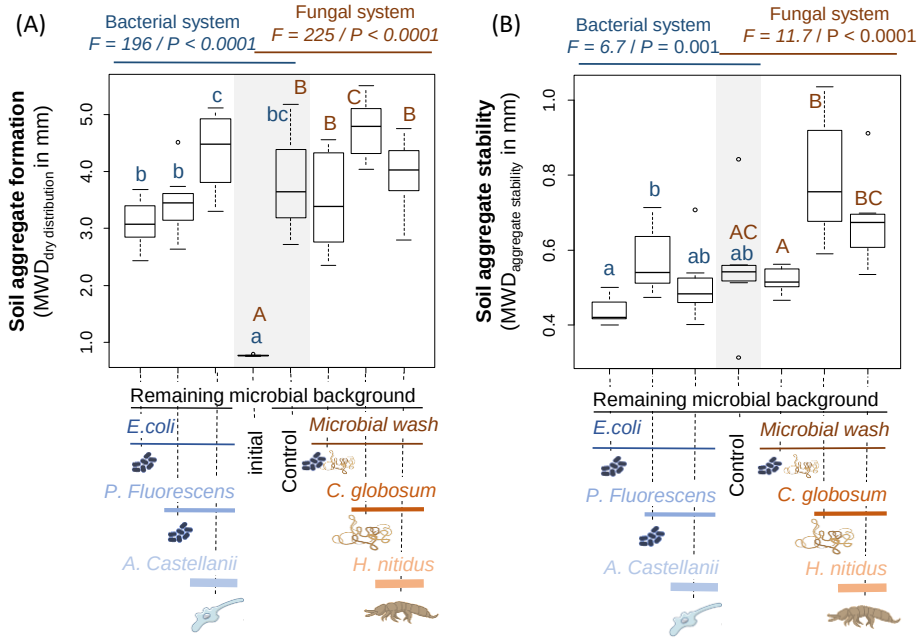

Figure 4 Effect of bacterial and fungal consumer - prey inoculations on (A) soil aggregate formation and (B) soil aggregate stability. Differences between treatments were ~~tested~~ analysed separately ~~within~~ for the bacterial and fungal systems using GLS models followed by ANOVA and post-hoc Tukey tests. Letters (blue lowercase for bacterial system and brown capital for fungal system) indicate significant differences between means according to Tukey tests. Grey background indicates control treatments common to bacterial and fungal systems. ***$P < 0.001$; **$P < 0.01$.

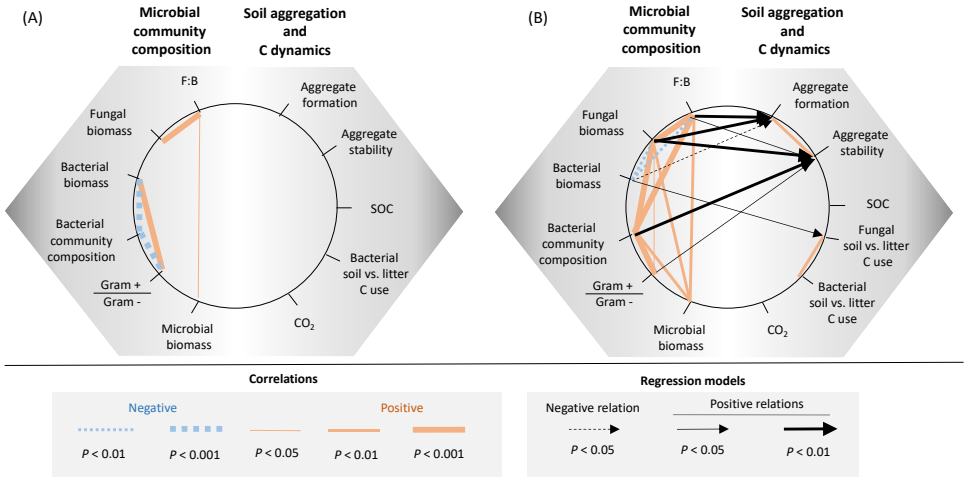

Figure 5 Relationships between microbial community composition and soil aggregation and C dynamics in the (A) bacterial-based and (B) fungal-based systems. Coloured lines indicate signification correlations (Spearman) among descriptors of microbial community composition and soil functioning; for details of the statistical parameters see Table A1 and A2. Black arrows indicate~~s~~ significant relationships between soil functioning variables and descriptors of the microbial community composition ~~tested~~ analysed by ~~simple~~ generalized linear models; for details of the statistical parameters see Table A3. Only significant relationships are displayed.

Table A1 Correlation matrix among descriptors of microbial community. Spearman's correlation coefficients (ρ) for the bacterial-based system (underlined letters) and the fungal-based system (bold letters).

	F:B	Fungal biomass	Bacterial biomass	Bacterial community composition	Gram + / Gram -	Microbial biomass
F:B	-	0.79 *P* < 0.00019***	- 0.32n30.s (*P* = 0.097)	0.31n.s (*P* = 0.09)	- 0.25 *P* = 0.16n.s	0.39 *P* = 0.03*
Fungal biomass	**0.99** ***P* < 0.0001***	-	0.22n24 *P* = 0.17.s	- 0.13 *P* = 0.5n.s	0.16 *P* = 0.4n.s	0.31n.s (*P* = 0.08)
Bacterial biomass	**- 0.64*** *P* = 0.0003**	**- 0.56** ***P* = 0.002****	-	- 0.7169 *P* < 0.0001***	0.58 *P* = 0.0007***	0.14n.s032 *P* = 0.9
Bacterial community composition	**0.71** ***P* < 0.0001***	**0.75** ***P* < 0.0001***	**- 0.29** ***P* = 0.12n.s**	-	- 0.74 P < 0.0001***	0.29 *P* = 0.1n.s
Gram + / Gram -	**0.29** ***P* = 0.13n.s**	**0.39** ***P* = 0.04***	**0.31** ***P* = 0.09n.s**	**0.71** ***P* < 0.0001***	-	0.06 *P* = 0.7n.s
Microbial biomass	**0.56** ***P* = 0.002****	**0.54** ***P* = 0.0034****	**- 0.32n.s** (***P* = 0.09**)	**0.55** ***P* = 0.002****	**0.28** ***P* = 0.14n.s**	-

***P < 0.001; **P < 0.01; *P < 0.5; n.s P > 0.5

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Table A2 Correlation matrix among variables describing soil aggregation and overall C use. Spearman's correlation coefficients (ρ) for the bacterial-based system (underlined letters) and the fungal-based system (bold letters). NA indicates not applicable.

	Aggregate formation	Aggregate stability	Fungal soil C use	Bacterial soil C use	CO_2	SOC
Aggregate formation	-	0.16 *P* = 0.4n.s	NA	0.07 *P* = 0.7n.s	- 0.12 *P* = 0.5n.s	- 0.07 *P* = 0.7n.s
Aggregate stability	**0.46** ***P* = 0.009****	-		- 0.34n.s (*P* = 0.06)	0.21 *P* = 0.3n.s	0.2 *P* = 0.2n.s
Fungal soil C use	**- 0.38n.s** (***P* = 0.06**)	**-0.15** ***P* = 0.5n.s**	-		NA	NA
Bacterial soil C use	**- 0.09** ***P* = 0.6n.s**	**- 0.07** ***P* = 0.7n.s**	**0.59** ***P* = 0.003****	-	- 0.10 *P* = 0.6n.s	- 0.03 *P* = 0.9n.s
CO_2	**- 0.13** ***P* = 0.5n.s**	**0.11** ***P* = 0.5n.s**	**- 0.03** ***P* = 0.9n.s**	**- 0.09** ***P* = 0.6n.s**	-	- 0.11 *P* = 0.5
SOC	**0.13** ***P* = 0.4n.s**	**- 0.05** ***P* = 0.8n.s**	**- 0.03** ***P* = 0.8n.s**	**- 0.07** ***P* = 0.7n.s**	**0.04** ***P* = 0.8n.s**	-

**$P < 0.01$; n.s $P > 0.5$

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Table A3 Simple linear regressions between soil structure, C dynamics and descriptors of soil microbial community composition. Relationships were tested using simple generalised linear models (GLM); only significant relations are displayed.

		Statistical parameters			
	Explanatory variables	R^2	*P*	Slope	ΔAIC
Fungal system					
Aggregate formation					
	Fungal biomass	0.29	0.002**	+	0
	F:B ratio	0.26	0.004**	+	1.2
	Bacterial biomass	0.18	0.02*	-	4.4
Aggregate stability					
	Bacterial community composition	0.28	0.002**	+	0
	Fungal biomass	0.24	0.007**	+	1.7
	F:B ratio	0.18	0.02*	+	3.9
	Gram + / Gram -	0.16	0.03*	+	4.6
Fungal soil vs. litter C use					
	Bacterial biomass	0.22	0.02*	+	-

**$P < 0.01$; *$P < 0.5$