

Running Head: Effects of herbivores and ants on litter decomposition

**A facilitation between large herbivores and ants accelerates litter decomposition by
 modifying soil micro-environmental conditions**

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Abstract

1. Large herbivores and insects commonly coexist and play important functional roles in grassland ecosystems. The interactive effects of these two animal groups in shaping ecosystem processes and functioning are poorly understood. In a semi-arid grassland of northeastern China, we previously found a reciprocal facilitation between large herbivores (cattle; *Bos tarurs*) and ants: cattle grazing led to a two-fold increase in ant mound abundance compared with ungrazed sites, while the presence of ant mounds, in turn, increased the foraging of cattle during the peak of the growing season.

2. Here, by using a large-scale, 4-year (2010-2013) manipulative experiment, we further investigated how such a facilitation between large herbivores and ants can affect a key ecosystem process, litter decomposition. Using a set of small-scale reciprocal translocation litterbag experiments, we separated the effects of litter quality and soil micro-environmental factors altered by cattle and ants on litter decomposition rates.

3. A significant interaction between the experimental factors, cattle grazing and ant presence, showed that litter decomposition rate was at the highest levels when both cattle and ants were present, with only a small impact when each was present on its own. Mechanistically, cattle and ants exerted limited effects on litter quality (litter C:N ratio). However, these animals greatly altered soil micro-environments by increasing soil N availability, which in turn increased soil microbial biomass and accelerated decomposition process.

4. *Synthesis.* Our results demonstrate how positive interactions between two groups of animals, large herbivores and ants, can affect decomposition rates, with important consequences for ecosystem carbon and nutrient cycling. Large herbivores, either domestic or wild, often coexist and interact frequently with a diverse of other fauna in terrestrial ecosystems. Assessing their

interactive effects will help us to better understand their role in shaping ecosystem processes and functioning with important management implications.

KEYWORDS

Facilitation; litter decomposition; herbivores; ants; soil micro-environment.

1. INTRODUCTION

Ecosystems harbor numerous species that interact directly and indirectly with each other (Ohgushi 2005). Ecosystem functions are regulated by the concert of these complex interactions. In studies of animal communities, there is increasing awareness that the outcome often depends on the way of how species interact with each other, either among closely related (Odadi, Karachi, Abdulrazak, & Young, 2011; Wang et al. 2019) or distantly related groups (Huntzinger, Karban, & Cushman, 2008; Risch et al., 2015). Interactive effects occur when the joint effect is significantly greater (or significantly less) than the sum of the individual impacts. Such effects are common in ecosystems and important for maintaining community structure and ecosystem functions (Davidson et al., 2010; Clark, Coupe, Bork, & Cahill, 2012; Risch et al., 2015; Wang et al. 2019, Zhong et al. 2021). Nevertheless, understanding of the mechanisms behind such interactive effects remains incomplete.

Coincidental with the appreciation of interactive effects, there has also been a realization that many interactions among animal species involve facilitations (Bertness, & Callaway, 1994; Bruno, Stachowicz, & Bertness, 2003; Ohgushi, 2005). It is suggested that animal species can indirectly benefit each other via at least three mechanisms. First, one animal species can indirectly increase the quality and quantity of food resources for other species (Arsenault, & Owen-Smith, 2002). Second, one animal species can act as “ecosystem engineer” and modify the

habitat structure and provide more shelters or refuges for other co-occurring animals (Jones, Lawton, & Shachak, 1994, 1997). Finally, when species share a same predator or competitor, the activities of one animal species may reduce the predation risk or competition for the second animal species (Holt, & Lawton, 1994; Young, Palmer, & Gadd, 2005). The facilitative interactions between different animal species have been increasingly documented in a variety of ecosystems, including temperate grasslands (Zhong et al., 2014), tropical savanna (Arsenault, & Owen-Smith, 2002; Young, Palmer, & Gadd, 2005), coastal dune ecosystem (Huntzinger, Karban, & Cushman, 2008), and temperate forests (Olofsson, & Strengbom, 2000; Ohgushi, 2005).

In semi-arid grasslands of northeastern China, our previous study found a facilitative interaction between large domestic herbivores (cattle, *Bos taurus*) and ants (e.g. *Lasius* spp. and *Formica* spp.) (Li et al. 2018). Cattle grazing significantly increased ant abundance by nearly twofold, whereas the presence of ants increased the foraging of cattle during the peak of the growing season. Cattle facilitated ants via ecosystem engineering by reducing plant litter accumulation and allowing more light to reach the soil surface that favour ants. Ants facilitated cattle by increasing resource quality for cattle: ants increased soil N availability that in turn increased the quality (N content) and quantity (biomass) of host food plants for cattle (Li et al., 2018). Facilitative interactions between animal species can lead to increases in the individual performance and/or population abundance of the interacting species (Bruno, Stachowicz, & Bertness, 2003), potentially strengthening the effect of species on ecosystem structure and functioning.

Large herbivores and ants are two important components of grassland ecosystems, where they commonly coexist and interact with each other. Although individual ant body sizes are small, the overall biomass of ants globally can be equal or even exceed wild terrestrial vertebrates or

livestock, with important consequences for ecosystem functioning (Del Toro, Ribbons, & Pelini, 2012; Tuma, Eggleton, & Fayle, 2020). One of the ecosystem processes that can be influenced by both large herbivores and ants is litter decomposition. These organisms can potentially influence decomposition in several ways. By their foraging, excreting, and trampling activities, large herbivores can alter soil nutrient availability (Augustine, McNaughton, & Frank, 2003; Risch et al., 2015) and plant nutrient concentrations (Ritchie, Tilman, & Knops, 1998; Fornara, & Du Toit, 2008), which in turn affect litter quality. Generally, litter with high nitrogen (N) concentration and low carbon:nitrogen (C:N) ratio and lignin concentration will benefit activity and biomass of soil microorganisms, and will decompose faster (Freschet, Aerts, & Cornelissen, 2012; Bradford et al., 2017). Large herbivores can also alter the soil micro-environment by reducing plant and litter cover, which can increase soil temperature and evaporation, reduce soil moisture, and alter soil pH (Bardgett, Wardle, & Yeates, 1998; Penner, & Frank, 2019). Higher soil moisture and temperature will commonly facilitate soil microbial activity and biomass (Wardle, 1992), which in turn accelerate litter decomposition (Ochoa-Hueso et al., 2019).

Ants may exert similar effects as large herbivores on soil micro-environmental conditions (e.g. increase soil N availability) by their nest-building and excretion activities (Holec, & Frouz, 2006; Cammeraat, & Risch, 2008; Evans, Dawes, Ward, & Lo, 2011), which in turn lead to shifts in soil microbial biomass and activity, and thus affect litter decomposition. During the past decades, independent effects of herbivores and ants on plant litter decomposition have been reported (Stadler, Schramm, & Kalbitz, 2006; Risch, Jurgensen, & Frank, 2007; Song et al., 2017; Prather, Strickland, & Laws, 2017). However, large herbivores and ants interact in ecosystem and may have synergistic effects on litter decomposition, with their net effects being context-dependent and determined by their abundance and the directions and strengths of

interspecific interactions. Until recently, few experiments have tended to explore the interactive effects of these animal species on litter decomposition rate.

Here, based on the facilitative interaction between cattle and ants we found previously (Li et al., 2018), we further assessed how their positive interactive effects can affect litter decomposition rates of a dominant grass species, *Leymus chinensis* in our system. We addressed the following core questions: (1) Do cattle and ants alone have a positive effect on litter quality, soil micro-environmental conditions, and decomposition rates? (2) Does the combined, facilitative interaction between cattle and ants have a larger positive effect on litter quality, soil micro-environmental conditions, soil microbial biomass, and decomposition rates compared to either animal group alone? And (3) can changes in litter decomposition be explained by cattle and ant induced alterations of the litter quality or soil micro-environmental conditions? To address these questions, we established a large-scale, 4-year (2010-2013) field experiment to examine how cattle and ants can alone and in combination affect litter quality (C:N), soil micro-environmental conditions, soil microbial biomass, and litter decomposition rates. To disentangle the relative effects of the changes in litter quality and soil micro-environmental conditions, we further conducted a set of small-scale reciprocal translocation litterbag experiments. We expected that cattle and ants alone have a positive effect on soil micro-environmental conditions and litter quality, which in turn will increase soil microbial biomass and thus litter decomposition rates. Moreover, we expected that cattle and ants together will accelerate litter decomposition more than when occurring alone, given the facilitative interactions between these two animals species (Li et al., 2018).

2. MATERIALS AND METHODS

2.1. Study site and background

The study was conducted in a semi-arid low elevation grassland, Jilin Province, China (44°36' N, 123°31' E, 300-500 m elevation). Mean annual precipitation in this area is approximately 350 ± 12 mm with about 70% falling during the growing season (May to October). Fires are infrequent and localized. The soil is nutrient-poor with available N concentration ranging from 15.4 to 27.6 mg/kg, and available phosphorus (P) concentration ranging from 2.6 to 5.9 mg/kg. The vegetation is dominated by the perennial grass *L. chinensis*, which accounts for > 50% of the total plant biomass (Liu et al. 2015a). Other frequently found plant species are *Calamagrostis epigeios* (grass), *Melilotus suaveolens* (legume) and *Kalimeris integrifolia* and *Artemisia scoparia* (forbs) (Liu et al., 2015a). Ants are common and abundant with *Lasius flavus*, *Formica sanguinea*, *F. candida*, and *Tetramorium caespitum* accounting for more than 60% of all ant individuals. Ant mound density is approximately 1 to 2 mounds per 1 m². The base diameter of these mounds ranges from 5 to 60 cm. We worked with all the ant species present in the study site (henceforth “ants”). The site has a long history of low-intensity livestock grazing (cattle and sheep) and mowing since 1980, but it was fenced in 2005 to protect against human disturbances (e.g. no grazing and mowing).

2.2. The large-scale cattle and ant manipulation experiment

In June 2009, we established six replicate (blocks) paired plots of 50 × 50 m. We randomly assigned one plot to cattle grazing (grazed), while the other as an ungrazed treatment (ungrazed). Distance between blocks ranged from 150 to 300 m, and the distance between the two plots in a block was on average 30 m. In each plot we randomly established eight 3 × 3 m subplots, separated by about 7 m. Four of these eight subplots within each grazed and ungrazed plot were

randomly assigned to the ant suppression treatment (no ant), while the other four were left unmanipulated (ant). Thus, we had four experimental treatments in a fully crossed 2×2 nested design, i.e. grazed + no ant, grazed + ant, ungrazed + no ant, and ungrazed + ant (see Fig. S1).

The six grazed-plots were grazed by cattle at a light to moderate intensity (less than 50% of aboveground plant biomass consumed by cattle) from June to September each year (2010 to 2013), a recommended grazing intensity by local governments. For the four ant suppression subplots within each plot, we repeatedly placed 10 g of poisonous ant bait (Jingkang Ant Bait Granules, Lekang Technology, Beijing, China) around each active ant nest each year from June to August (once each month). This period is the active period of ants each year. The main active ingredients of the ant bait are 0.45% Tetramethrin and 0.02% Alpha cypermethrin. The ant bait is specifically designed to kill ants and their colonies and has been used successfully to reduce ant populations in the region. Our ant led to a 96% reduction in total active ant nest densities in this study, with 2.71 (s.e. 0.48) ant nests m^{-2} in ant subplots compared to 0.07 (s.e. 0.01) ant nests m^{-2} in the no ant subplots (Li et al., 2018).

2.3. Pre-treatment conditions

We measured pre-treatment conditions at the peak of the growing season in August 2009, one year prior to introducing our treatments. We sampled plant community characteristics, soil properties, microclimate and ant abundance within the eight 3×3 m subplots in each plot. We found no significant differences for these variables between the plots (see Supplementary Materials in Li et al., 2018).

2.4. Effects of cattle and ants on litter decomposition, litter quality, and soil

micro-environmental conditions

During growing seasons (May to October) of 2013, we assessed how cattle grazing and ant suppression affected litter decomposition, litter quality, soil micro-environments, and soil microbial biomass. On April 28, 2013, we randomly harvested *L. chinensis* litter from all 3 m × 3 m subplots of the four experimental treatments (i.e. grazed + no ant, grazed + ant, ungrazed + no ant, and ungrazed + ant). To avoid edge effects, we established a 1 m buffer in the subplots, where we did not collect plant material. To make the litter collection homogenous, we selected approximately 25-28 cm long plants with 4-6 leaves attached to the stem. We dried the plant litter for 48 h at 65 °C and weighed it.

We put 10 g ± 0.2 g of the collected litter into 20 cm × 10 cm nylon-bags with a mesh size of 2 mm to assess decomposition rates. On May 3, 2013, we randomly placed two litter bags in each of the 96 3 m × 3 m subplots (192 litterbags in total). All litterbags were fixed by nails on top of the soil surface. Each litterbag was covered by a gridded metal cage (15 cm height × 30 cm length × 20 cm width) to prevent cattle trampling from affecting litter decomposition in the grazed plots. Cages were also placed in the ungrazed plots to create consistent conditions and avoid a potential bias. Cages were anchored to the ground by four steel legs (Fig. S1). The gridded cages allowed plants to grow through, and feces and urine of cattle to reach the soils (Fig. S1). Our preliminary investigations showed that these metal cages did not significantly alter the microclimate conditions and litter decomposition rates in the systems (Table S3). Litterbags were collected on October 30, 2013, 180 days after deployment. Each retrieved litterbag was emptied, the remaining litter was washed to clear external soil, oven-dried at 65 °C for 72 hours and weighed. Litter mass remaining (%) was calculated as (dry litter mass remain at the sampling date / dry litter mass at the initial date) × 100.

We measured the initial quality from the litter collected from the four experimental treatments on April 28, 2013. For this purpose, we dried the plant litter for 48 h at 65 °C. Carbon (C) and N concentration of litter was then analyzed by an automated element analyzer (Vario EL cube, Elementar Analysensysteme GmbH, Hanau, Germany), after grinding the samples (0.5 mm mesh, Wiley mill).

In mid August 2013, we collected data to address how our treatments affected the soil-environmental conditions and soil microbial biomass. We measured soil pH, moisture, and temperature by using a handheld multi-function soil parameter reader (OSA-1, OUSU Technology, Hebei, China), taking readings from three random locations at 5 cm depth within the inner 2 × 2 m area of each of the subplots. We used a 4 cm diameter soil auger to randomly collected three 0-5 cm soil samples from each subplot to measured soil nutrients and microbial biomass C. The three samples per subplot were pooled and homogenized. Soil total C was determined using as described for plants, after the air-dried soil samples were fine ground and sieved through a 0.5 mm mesh. From the same soil samples, a 10 g subsample was extracted with 70 mL 2 mol L⁻¹ KCl. Extracts were frozen at 20 °C for analysis of NH₄⁺ and NO₃⁻ content by continuous flow analyzer (Alliance Flow Analyzer; Futura, Frépillon, France). Total available N was the sum of NH₄⁺ and NO₃⁻ concentrations. Soil microbial biomass C (mg C g⁻¹ soil dry mass) were measured using the chloroform fumigation extraction method.

2.5. Additional small-scale reciprocal litterbag experiments

During growing seasons of 2013, we also conducted two small-scale reciprocal translocation litterbag experiments to disentangle the effects of the changes in litter quality and soil micro-environment as altered by cattle and ants on decomposition process.

Ant and cattle effects on litter quality and decomposition. On May 5, 2013, we collected *L. chinensis* litter from the 3 m × 3 m subplots of the four experimental treatments in the large-scale cattle and ant manipulation experiment. After drying litter at 65 °C to constant weight, 10 g ± 0.2 g of shoot biomass were filled into 20 cm × 10 cm nylon-bags (mesh size 2 mm). On May 10, 2013, 15 litterbags from each of the four treatments (total of 60) were randomly deployed to 5 m × 5 m plot that was protected from human disturbance (e.g., no grazing and mowing) for over 10 years. Distance between litterbags was about 20 cm. All litterbags were fixed by nails on top of the soil surface. Litterbags were collected on October 31, 2013, 175 days after litter exposure. Each litterbag was carefully emptied and washed clear of external soil. Litter were oven-dried at 65 °C for 72 hours and weighed. Again, we calculated the mass remaining as the difference between the initial and final litter dry mass.

Ant and cattle effects on soil micro-environments and decomposition. On April 20, 2013, we collected *L. chinensis* litter with the similar quality from the same protected area as above (Table S4). We used the same methods described above to treat litter and make litter bags. On April 25, 2013, we randomly placed two litterbags in each of the 96 3 m × 3 m subplots (192 litterbags in total), and collected them on October 30, 2013, 180 days after litter exposure. Litterbags were fixed to the soil surface and covered by a gridded metal cage to prevent cattle trampling. Mass remaining of litter was calculated using the same method above.

2.6. Statistical analyses

We averaged each variable described above for the four replicate 3 m × 3 m subplots within each ungrazed and grazed plot for statistical analyses leading to a total 24 independent samples. All data were assessed for normality and analysed using the open source software R version

4.0.1 (R core team, 2020). We used linear mixed effects models (LMMs) provided by the nlme package (Pinheiro, Bates, DebRoy, & Sarkar, 2020) to test for effects of cattle grazing and ant suppression on litter decomposition rates, litter quality, and soil micro-environmental conditions, and soil microbial biomass. In all models, cattle presence (grazed or ungrazed), ant presence (ant or no ant), and their interaction were treated as fixed factors, with block (6 blocks) included as a random factor. For testing for the impact on C:N ratio, we used a gls model and VarIdent provided by the nlme package to account for variance heterogeneity in effect sizes between treatment groups. For all response variables, we were also interested in comparing the individual and combined effects of cattle grazing and ant suppression treatments. Therefore, in cases where the cattle \times ant interaction was significant ($P < 0.05$), we tested for post-hoc differences among treatment means using Tukey's multiple comparison provide by the package lsmeans (Lenth, 2016). Given that no soil micro-environmental factors but soil N availability was significantly affected by cattle and ant activities (see Results below), we further evaluated the potential influence of changes in soil available N on soil microbial biomass, and the influences of changes in soil microbial biomass on litter decomposition using a linear model.

3. RESULTS

3.1. Effects of cattle and ants on decomposition, litter quality, soil micro-environmental conditions, and soil microbial biomass

A significant interaction between cattle grazing and ant presence showed that litter decomposition was at highest levels when both cattle and ants were present, with only a small impact when each was present on its own (cattle \times ants: $F_{1,15} = 11.12$, $P = 0.005$). Post-hoc Tukey's test showed that when cattle and ants were together (grazed + ant), litter decomposition

was significantly higher compared any of the other treatments (i.e. grazed + no ant, ungrazed + no ant, and ungrazed + ant) (Fig. 1).

Neither cattle grazing, ant suppression, nor their interaction had significant impacts on litter quality, as presented by C concentration, N concentration, and C:N of plant litter (Fig. S2, Table S1). In contrast, cattle and ants exerted strong effects on soil micro-environmental conditions (Fig. 2). Cattle increased soil temperature by 3% ($F_{1,15} = 4.50$, $P = 0.049$, Fig. 2c), whereas ants did not affect this variable ($F_{1,15} = 0.80$, $P = 0.385$, Table S2). Cattle increased soil available N and microbial biomass by 10% and 27%, respectively (Table S2, Fig. 2e, f). Similarly, ants increased soil available N and microbial biomass by 12% and 22%, respectively (Table S2, Fig. 2d, e, f). Moreover, a significant interaction between cattle and ant presence for both soil available N and microbial biomass showed synergistic effects between both factors (Table S2, Fig. 2e, f). Post-hoc Tukey's test showed that both soil available N and microbial biomass was significantly higher in the grazed + ant subplots compared to the other treatments (Fig. 2e, f). Neither cattle grazing, ant suppression, nor their interaction had significant impacts on soil pH, moisture, and soil C concentration (Fig. 2a, b, d, Table S2).

Linear regression analyses showed that soil total microbial biomass was positively related to soil available N ($R^2 = 0.69$, $F_{1,22} = 48.33$, $P < 0.001$, Fig. 3a), and litter mass remaining was negatively related to soil total microbial biomass ($R^2 = 0.47$, $F_{1,22} = 19.57$, $P < 0.001$, Fig. 3b) in the subplots of the large-scale cattle and ants manipulated experiments.

3.2. Effects of cattle and ants induced changes in soil micro-environmental conditions that affected decomposition

As litter quality did not differ between our treatment plots (Fig S2, Table S1), it was not

surprising that we found no difference in the decomposition of plant litter when placed into an undisturbed control site (Fig 4a). Similarly, when placing litter from the undisturbed control site (same litter quality) into the experimental plots, we found that litter decomposition was, again, significantly affected by the interaction between cattle and ants (cattle \times ants: $F_{1,15} = 13.93$, $P = 0.002$). Tukey's test showed that litter decomposition was significantly faster in the grazed + ant subplots compared to the grazed + no ant, ungrazed + no ant, and ungrazed + ant subplots (Fig. 4b).

4. DISCUSSION

Our study investigated the separate and interactive effects of large herbivores and ants on litter decomposition in a temperate grassland ecosystem. Consistent with our expectations, we found that a facilitative interaction between cattle and ants led to a much faster litter decomposition compared to either group of animals alone. The interactive effects of cattle and ant presence on litter decomposition appear to be mediated by the changes in soil micro-environmental conditions (e.g. soil N availability), rather than litter quality (e.g. C:N) induced by these animals. Our results highlight that interactive effects between large herbivores and smaller insects can shape ecosystem structure and functioning.

4.1. The interactive effects of cattle and ants on litter decomposition

We found the strongest impacts of cattle and ants on litter decomposition when they co-occurred compared to when each occurred alone (Fig. 1). We are aware of only two experimental studies that evaluated the combined impacts of functionally different animal groups (e.g. large, medium and small mammals, and invertebrates) on litter decomposition. One was conducted in the subalpine habitat in the Swiss National Park, but no interactive effects of

vertebrate (e.g. large ungulates) and invertebrate animals (e.g. insects) on litter decomposition were reported (Haynes et al., 2014). However, another study performed in the hardwood forests in North America found that a positive interaction between white-tailed deer (*Odocoileus virginianus*) and invasive earthworms (e.g. *Aporrectodea tuberculata*) occurred, which led to more rapid litter decomposition compared to either group of animal present alone (Mahon, Fisk, & Crist, 2020). Consistent with the results from that study, the large combined impact of cattle and ants on litter decomposition in our experiment was likely a result of facilitative interactions between them. In a previous study, Li et al. (2018) found a nearly two-fold increase in ant abundance in plots grazed by cattle, probably because cattle grazing reduced plant litter accumulation and allowing more light to reach the soil surface that favor ants. The increases in ant abundance, in turn enhanced soil N availability and increased the quality and quantity of host plants for cattle. Such positive interactions appear to be common between large herbivores and other animal groups worldwide. For example, large herbivores were shown to facilitate grasshoppers (e.g. *Euchorthippus cheui*), gall-forming insects (e.g. *Pontania glabrifrons*), and caterpillars (e.g. *Platyrepia virginalis*) in temperate grassland (Zhong et al., 2014), arctic tundra (Olofsson, & Strengbom, 2002), and coastal dune ecosystem (Huntzinger, Karban, & Cushman, 2008). Large herbivores also facilitated small mammals like prairie dogs (*Cynomys* spp.) and lizards (*Lygodactylus keniensis*) in many grassland ecosystems (Davidson et al., 2010; Pringle, Palmer, Goheen, McCauley, & Keesing, 2011). These positive interactions between animals commonly lead to the increases in individual performance and/or population abundance of the interacting species, potentially strengthen their impacts on ecosystem structure and functioning. In our study, when cattle and ants were present together, the increases in ant abundance and the more frequent grazing activities of cattle (Li et al., 2018) led to a significantly higher soil N

availability and microbial biomass in the habitats compared to the presence of cattle and ants alone (Fig. 2e, f), with important consequences for litter decomposition.

4.2. Mechanisms of how cattle and ants affect litter decomposition

Litter quality and soil micro-environmental conditions are two key determinants of soil microbial biomass and activities, which in turn affect litter decomposition (Prescott, 2010). Cattle and ants did not affect litter quality in our study (Fig. S2, Table S1). The limited responses of litter quality to both cattle and ant presence could be due to the relatively short duration (3 years) of the experimental treatments and the relatively low N concentration of grass litter itself (Fig. S2b).

In contrast, cattle and ants affected soil micro-environmental conditions, particularly soil N availability (Fig. 2e). The positive effects of large herbivores on soil available N have been widely documented for a range of grassland ecosystems (Ritchie, Tilman, & Knops, 1998; Frank et al. 2018). Large herbivores can increase soil nutrient concentrations via two primary pathways: First, herbivory can change the productivity and composition of plant communities and thus increase litter quantity and quality that enters the belowground subsystem, which in turn can positively affect soil nutrient concentrations (Bardgett, Wardle, & Yeates, 1998; Augustine, McNaughton, & Frank, 2003; Frank et al., 2018). Second, feces and urine deposition by large herbivores can accelerate nutrient cycling and increase soil N concentrations (Frank et al., 2018). In our ecosystems, the relative importance of these two pathways is difficult to distinguish, as they often act simultaneously and exert combined effects on soil nutrient concentrations (Liu et al., 2015b, 2018).

Similarly to cattle, many studies also reported positive effects of ants on soil fertility. In a recent meta-analysis of 106 studies, Farji-Brener & Werenkraut (2017) found that the presence of ants commonly lead to a higher soil nutrient concentration in their habitats, and that ant nests

improve soil fertility mainly through the accumulation of waste materials. In our study, one of the dominant ant groups, *Formica* spp. (e.g. *F. sanguinea*, *F. candida*) are omnivorous. These ants eat all plant litter and insects they can find and use *L. chinensis* litter as mound-building materials (X. Li, *field observations*). Also, transporting other types of organic matter (e.g. high-protein plant tissues, seeds, feces) by the ants to their mounds may have led to higher soil N availability (Wu, Lu, Wu, & Yin, 2010). In contrast, another ant species such as *Lasius* spp. (e.g. *L. niger*, *L. flavus*), lives primarily underground and often feed on honeydew excreted by leaf and root aphids (Holec, & Frouz, 2006). These ant species were reported to lower soil total N concentrations and available N (e.g. NH_4^+ and NO_3^-) (Holec, & Frouz, 2006). Given the opposed effects of the two ant groups on soil fertility, the positive net effects on soil N availability observed on our study points towards a larger impact of *Formica* spp. ants in our system.

The increases in soil N availability caused by cattle and ants also benefited soil microbial biomass, which in turn accelerated litter decomposition (Fig. 3). However, it is surprising that we found a positive effect of soil available N on soil microbial biomass and litter decomposition, as many other studies reported lower soil microbial activities and abundance and litter decomposition rates when more N was available in the soil (Craine, Morrow, & Fierer, 2007; Hobbie, 2008). High soil N-availability can constrain decomposition rates by i) direct suppression of ligninolytic enzyme activity (Ramirez, Craine, & Fierer, 2012), ii) alleviation of microbial N requirements and therefore a reduction in microbial N-mining of recalcitrant compounds (e.g., lignin; Craine, Morrow, & Fierer, 2007; Talbot, & Treseder, 2012), and iii) shifting the microbial community composition (Carreiro, Sinsabaugh, Repert, & Parkhurst, 2000; Talbot, & Treseder, 2012). Nevertheless, some experiments found no relationship between soil N

availability and soil microbial biomass and decomposition rates (Carreiro, Sinsabaugh, Repert, & Parkhurst, 2000), whereas others have reported increased decomposition rates in relation to higher soil N concentrations (Hobbie, 2000). Further studies are needed to instigate the mechanisms underlying the observed positive effects of soil N availability on soil microbial biomass and decomposition rates in our ecosystems. In addition to soil N availability, cattle and ants may also affect soil microbial community composition by affecting other abiotic factors, including soil pH, moisture, and temperature (Wardle, 1992), and thus affecting litter decomposition rates. Cattle only marginally increased soil temperature by 3% (Fig. 2c), probably by their negative impacts on plant and litter cover that allowed more sunlight and solar radiation penetrated into the soil surface. A warmer habitat often benefits the activities and growth of microorganisms, and thus accelerates litter decomposition (Wardle, 1992). To what extent the increases in soil temperature contributed to the increases in soil microbial biomass and litter decomposition in the cattle grazing areas remains to be explored. In addition, given that cattle and ants exerted very limited effects on soil pH and moisture, these factors unlikely explained the changes in soil microbial biomass and litter decomposition in our study (Fig. 2a, b). Finally, large herbivores may also directly accelerate litter fragmentation and decomposition by trampling (Augustine, McNaughton, & Frank, 2003), potentially biasing the effects mediated by changes in soil micro-environment conditions and microbial biomass on decomposition that we detected. However, we omitted this direct effect by protecting the litterbags with cages in our study (see *Materials and methods*, Fig. S1).

5. CONCLUSIONS

We found that cattle and ants can jointly increase litter decomposition rates, with important

consequences for C and N cycling in grassland ecosystems. The facilitative interactions between cattle and ants exerted a larger impact on soil micro-environmental conditions, leading to even faster litter decomposition compared to when either group of animals was alone. It should be noted that, the directions and strengths of the interactions between large herbivores and ants often depend on the abundance of interacting animals involved (Tadey, & Farji-Berner, 2007). Further studies are needed to explore how ants can interact with livestock and how their interactive effects with livestock on ecosystems can be changed along different stocking rates. Large herbivores are widespread in many ecosystems, and coexist and interact with a diversity of other fauna (e.g. small mammals and insects), either antagonistically or synergistically, with unpredictable ecological consequences (van Klink et al. 2015). Given that both large herbivores and invertebrates are facing dramatic shifts in their abundance and distribution due to climate changes and human activities globally (Ripple et al., 2015; Sánchez-Bayo, & Wyckhuys, 2019), assessing their interactive effects with other animal groups may not only help us better understanding their role in shaping ecosystem structure and functioning, but may have important management implications.

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AUTHORS' CONTRIBUTIONS

X.L., and Z.Z. designed the research and wrote the draft manuscript. A.C.R., C.P., D.W., and Q.G. substantially revised the manuscript. G.L. and D.S. analysed data. X.L., Z.W. and N.H. performed the research and contributed to data collection. All authors edited the manuscript.

CONFLICT OF INTERST STATEMENT

All authors certify that they do not have any conflicts of interest to disclose.

ETHICS

All experimental procedures were carried out in accordance with the Law of the People's Republic of China on the Protection of Wildlife (1988).

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.s7h44j16f> (Li et al., 2021).

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FIGURE LEGENDS

Figure 1. Effects of cattle and ants on litter mass remaining of *L. chinensis* grass in the large-scale cattle and ant manipulated experiments. Presented are the median, the lower and upper quartiles at 25% and 75%, respectively, and the single values. Different letters above bars indicate significant differences among treatments, as assessed via post-hoc Tukey test at $P < 0.05$ used to evaluate the interaction between grazing and ants.

Figure 2. Effects of cattle and ants on soil micro-environment conditions in the large-scale cattle and ant manipulated experiments. (a) soil pH, (b) soil moisture, (c) soil temperature, (d) soil N concentration, (e) soil C:N, and (f) soil microbial biomass. Presented are the median, the lower and upper quartiles at 25% and 75%, respectively, and the single values. Different letters above bars indicate significant differences among treatments, as assessed via post-hoc Tukey test at $P < 0.05$ used to evaluate the interaction between grazing and ants.

Figure 3. Relationships between soil available N and soil microbial biomass (a) and between soil microbial biomass and litter mass remaining (b) in the large-scale cattle and ant manipulated experiments.

Figure 4. Effects of the changes in (a) litter quality and (b) soil micro-environment as altered by cattle and ants on decomposition in the additional small-scale reciprocal translocation litterbag experiments. Presented are the median, the lower and upper quartiles at 25% and 75%, respectively, and the single values. Different lower case letters above the treatments indicate

significant differences based on a Tukey test at $P < 0.05$.

Figure 1.

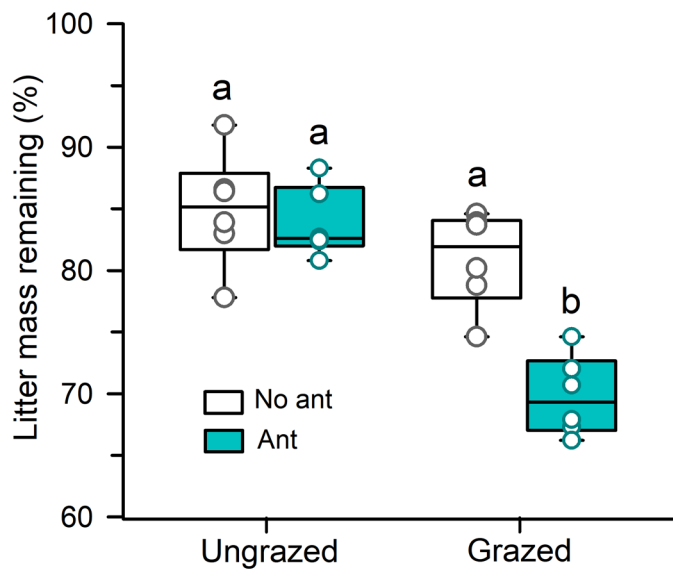


Figure 2.

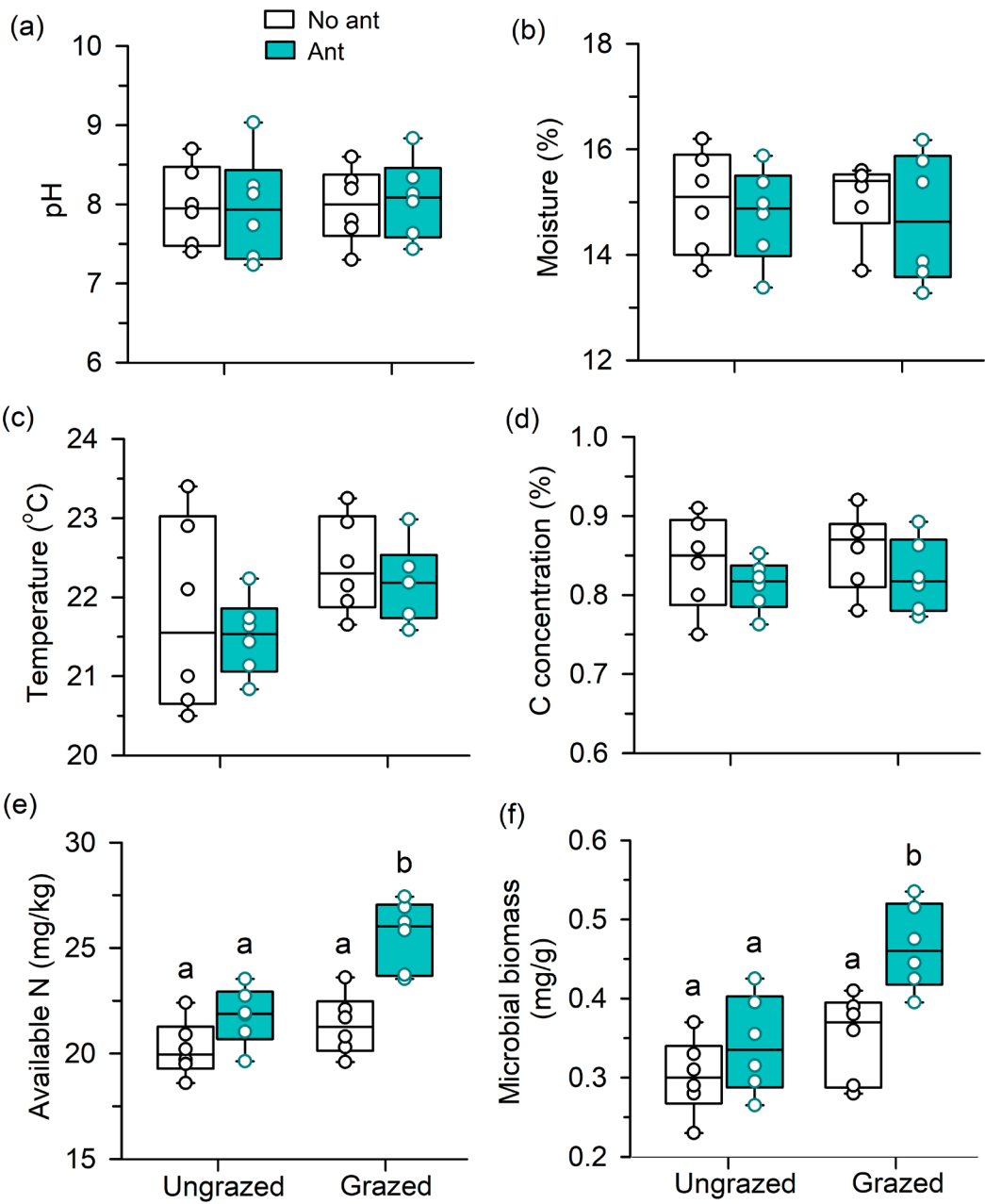


Figure 3.

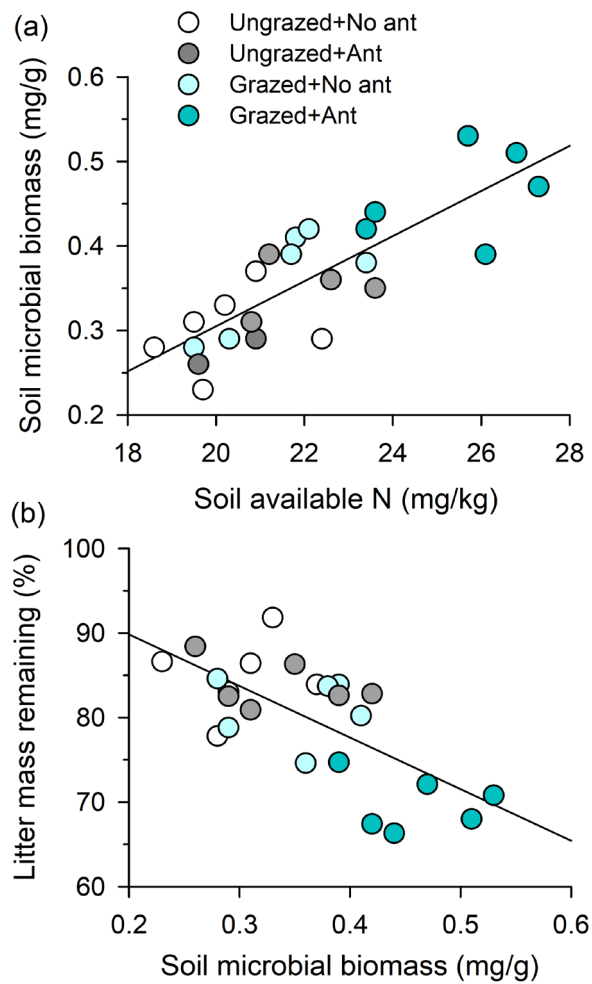


Figure 4.

