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Grasshopper herbivory immediately affects element cycling but not export rates in an N-limited grassland system

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Abstract. As a cause of ecosystem disturbances, phytophagous insects are known to directly influence the element and organic matter (OM) cycling in ecosystems by their defoliation and excretion activity. This study focuses on the interplay between short-term, insect herbivory, plant responses to feeding activity, rhizosphere processes, and belowground nutrient availability under nutrient-poor soil conditions. To test the effects of insect herbivory on OM and nutrient cycling in an N-limited pasture system, mesocosm laboratory experiments were conducted using *Dactylis glomerata* as common grass species and *Chorthippus dorsatus*, a widespread grasshopper species, to induce strong defoliating herbivory. $^{13}CO_2$ pulse labeling was used together with labeled ^{15}N feces to trace the fate of C in soil respiration at the beginning of herbivory, and of C and N in above- and belowground plant biomass, grasshopper, feces, bulk soil, soil microbial biomass, throughfall solutions, and soil solutions. Within five days, herbivory caused a reduction in aboveground grass biomass by about 34%. A linear mixed-effects model revealed that herbivory significantly increased total dissolved C and N amounts in throughfall solutions by a factor of 4–10 ($P < 0.05$) compared with the control. In total, 27.6% of the initially applied feces ^{15}N were translocated from the aboveground to the belowground system. A significant enrichment of ^{15}N in roots led to the assumption that feces-derived ^{15}N was rapidly taken up to compensate for the frass-related foliar N losses in light of N shortage. Soil microorganisms incorporated newly available ^{13}C; however, the total amount of soil microbial biomass remained unaffected, while the exploitative grass species rapidly sequestered resources to facilitate its regrowth after herbivory attack. Heavy herbivory by insects infesting *D. glomerata*-dominated, N-deficient grasslands remarkably impacted belowground nutrient cycling by an instant amplification of available nutrients, which led to an intensified nutrient competition between plants and soil microorganisms. Consequently, these competitive plant–soil microbe interactions accelerated N cycling and effectively retained herbivory-mediated C and N surplus release resulting in diminished N losses from the system. The study highlighted the overarching role of plant adaptations to in situ soil fertility in short-term ecosystem disturbances.

Key words: carbon allocation; *Chorthippus dorsatus*; *Dactylis glomerata*; ^{15}N; feces deposition; Hainich; isotope labeling; plant response; soil solution; ^{13}C; throughfall.

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Introduction

Insects play a pivotal role in grassland ecosystems, providing important ecological services as prey, predators, pollinators, consumers, and decomposers (Paterson and Sim 2000, Weisser and Siemann 2008). Under mass outbreak situations and due to massively intensified leaf-defoliating and sap-feeding activities, herbivorous insects directly and indirectly affect a variety of ecosystem processes and functions, hence establishing an ecosystem disturbance (Belovsky and Slade 2000, Michalzik and Stadler 2000, Chapman et al. 2003). In this context, Hunter (2001) pinpointed seven mechanisms by which insect herbivores modify nutrient and organic matter (OM) cycles. Three of these, encompassing direct inputs of frass (fecal material), insect cadavers, and green fall (green foliage fragments), have been demonstrated to alter the timing, amount, and quality of nutrient fluxes to the ground (Stadler et al. 2001, Le Mellec et al. 2011, Burghardt et al. 2018). Additionally, phytophagous insects enhance nutrient concentrations and OM contents in throughfall solution by boosted leaching from insect-damaged foliar biomass or through the dissolution of frass material (Hunter 2001, Lovett et al. 2002, Nitschke et al. 2015). Both processes facilitating the release of nutrients and energy previously bound in plants and therefore appear to be of particular importance for short-term changes in the rates of nutrient cycling due to the prompt availability to soil microorganisms and plants (Lovett and Ruesink 1995, Chapman et al. 2003).

The primary plant metabolism can be profoundly impacted by herbivore activity (Orians et al. 2011), creating an exogenous biotic stress factor for plants (Schulze et al. 2019), which alters the plant's capacity for resource assimilation and source–sink relationships to finally meet its metabolic demands (Schwachtje and Baldwin 2008). Carbon allocation to plant roots and rhizosphere microorganisms can be influenced by physical (e.g., reduction in aboveground biomass) and physiological plant responses (e.g., by the activation of chemical defense compounds due to salivary components; Dyer and Bokhari 1976). Response mechanisms encompass the activation of storage reserves, mainly derived from roots, to induce the production of chemical defense compounds and to stimulate aboveground regrowth and reproduction (Bokhari 1977, Holland and Detling 1990, Mackie-Dawson 1999). Besides changes in root architecture and photosynthetic capacity (Schwachtje and Baldwin 2008), a rapid allocation of resources from damaged tissue into storage organs can occur, as well as an increase in the plant's tolerance to herbivore attack by a so-called "induced resource sequestration" (Orians et al. 2011).

The belowground nutrient availability and metabolic activity of plants strongly depend on the photosynthetic activity (Craine et al. 1999, Kuzyakov and Gavrichkova 2010), exhibiting a close coupling between photosynthesis and rhizosphere processes on variable timescales. In their review, Kuzyakov and Gavrichkova (2010) reported mean time lags of 12 h between recently assimilated C of grass plants and maximum belowground CO_2 efflux from the rhizosphere. Hampered or imbalanced partitioning of photosynthates among sources (i.e., areas with sugar excess and low energy demand) and sinks (i.e., areas of high-energy demand encompassing actively growing tissues such as fine roots) can be the result of amplified plant energy demands after herbivore attack. Overall, this tends to decrease soil CO_2 efflux rates on a daily timescale (Craine et al. 1999).

However, such immediate reduction in photosynthates due to herbivory does not necessarily lead to decelerated root exudation rates thereafter. Amplified root exudations following a reduction in aboveground biomass are reported in previous studies (Dyer and Bokhari 1976, Holland et al. 1996, Paterson et al. 2003) and might be traced back to triggered belowground interactions between plants and soil microorganisms (Bardgett et al. 1998, Kuzyakov and Domanski 2000). Holland et al. (1996) revealed that this stimulation of soil microbial activity induced an accelerated decomposition of OM and to a subsequent release of available nutrients fostering the plant's repair and defense capabilities.

The review by Hunter (2001) emphasized that the effects of insect herbivory on nutrient dynamics vary in space and time being dependent on the environmental context, and hence resulting in study outcomes with opposing effects or interactions over time. For instance, in nutrient-rich ecosystems plant's resistance to herbivory can be

higher due to enhanced storage of chemical defense compounds, while under nutrient limitation tissues were found to be more susceptible to herbivory and subsequent decomposition with, for example, defoliated grasses releasing higher amounts of rhizodepositions under low N availability (Paterson and Sim 2000, Burghardt et al. 2018). Furthermore, a recovery from herbivore plant damage under low nutrient availability was found to be at the expense of stem growth compared with nutrient-rich soil conditions (Meyer 2000). Lovett et al. (2002) showed that ecosystems with a high N saturation have lower potentials to retain rapidly released N from feces leachates. Hence, in addition to plant species traits encompassing for instance growth-limiting factors (Ritchie et al. 1998), tissue N storage (Orians et al. 2011), or the interplay with soil microorganisms, which alter N immobilization rates (Lovett and Ruesink 1995), also soil properties appear to control the risks of herbivory-induced nutrient losses via soil leaching (Hunter 2001). As a result of different environmental conditions, substantial variation in, for example, N export between 0.00004% (Christenson et al. 2002) and 20% (Frost and Hunter 2004) of the deposed feces N can occur. All these investigations demonstrate the importance to consider the overall nutrient status of the investigated herbivory system.

To understand the initial mechanisms of phytophagous herbivory on plant response and C and N cycling, our present study focuses on short-term effects of plant–herbivore interactions in a grassland system under N-limited conditions. To meet the initial environmental field conditions of our experiment exhibiting an N-deficient and alkaline pasture soil, an exploitative perennial and widely common grass species *Dactylis glomerata* was selected. Analogous to the "fast cycle" concept, originally termed by McNaughton et al. (1988), the experiment was designed to cover the rapid responses of *D. glomerata*, soil microbes, and C and N cycling to grasshopper *Chorthippus dorsatus* herbivory inducing the input of easily available organic compounds through feces leaching and via throughfall deposition. To the best of our knowledge, this is the first mesocosm experiment with a well-developed root system of a one-year-old pasture grass to track herbivory effects under controlled environmental conditions by means of $^{13}CO_2$ pulse labeling (Kuzyakov and Gavrichkova 2010) and ^{15}N-enriched feces (Frost and Hunter 2007). In detail, we addressed two questions: (1) What are the initial implications of a severe grasshopper herbivory on above- and belowground C and N cycling in this grassland ecosystem? (2) What is the magnitude of changes in C and N quantity, release, and immobilization by different ecosystem strata? We hypothesize that herbivory-induced losses of foliar biomass cause a reduced translocation of C assimilates to the root system and hence affect belowground processes as indicated by altered soil respiration rates, amounts of microbial biomass, solution chemistry, and N uptake.

Methods

For all (pre- and key) experiments, mineral soil material was collected from a pasture site (51°06′ N, 10°24′ E) of the Hainich Critical Zone Exploratory (HCZE), which was established as an interdisciplinary research platform on subsurface biodiversity within the Collaborative Research Center (CRC), Aquadiva—Understanding the Links Between Surface and Subsurface Biogeosphere (Küsel et al. 2016). The soil contained 82% silt and 11% clay and was homogenized by sieving to 6 mm and mixed with washed gravel (30 mass%, 3 mm in diameter) to prevent stagnic soil water conditions. The slightly calcareous soil material was characterized by 1.34% C_{org}, 0.14% N_{tot}, and a $pH_{(H_2O)}$ of 6.7. Soil N-deficient conditions were obvious due to the low soil N content and the high C:N ratio (>30) of the aboveground grass biomass. Furthermore, the perennial grass species *D. glomerata* (L.), a highly common plant species in Central German pasture and grasslands, was chosen for all experiments. It exhibits the capability to exploit a wide range of soil nutrient availabilities, under normal to dry soil moisture conditions and from slightly acidic to alkaline soil pH (Beddows 1959, Poorter and Remkes 1990). Grown in the above described pasture soil, this grass increased its growth rapidly after amendment of N fertilizer. *D. glomerata* is a potential food plant for the selected grasshopper species *C. dorsatus* (G. Koehler, *personal communication*) that was caught on a grassland site near Jena.

Pre-experiments

As a prerequisite for conducting the key herbivory experiment, a deeper understanding was necessary with regard to the magnitude and temporal evolvement of the plant–soil system feedback to herbivory. Hence, we investigated the short-term response of above- and belowground C cycling due to grasshopper herbivory (*C. dorsatus*) on *D. glomerata* by real-time monitoring of the soil respiration rates ($^{12}CO_2$, $^{13}CO_2$) after the host plant was exposed to grasshoppers and $^{13}CO_2$ pulse labeling. Since the results of this pre-experiment formed the base for the methodological setup and temporal expenditure of the key experiment, we will report on the results already in this section. Furthermore, a second pre-experiment was performed to generate sufficient amounts of ^{15}N-labeled grasshopper feces for the key experiment.

Belowground response to insect herbivory and $^{13}CO_2$ pulse labeling.—For the pre-experiment, eight medium-sized (18 L volume) mesocosms, custom-made by transparent makrolon polycarbonate (100 cm height, 17 cm in diameter), were used. The mesocosms consisted of five individual parts: a round bottom plate with an outlet port, two cylinders (bottom unit 30 cm, top unit 70 cm height with six ports each), a perforated plate (2 cm thick) that connects the two cylinders, and a cover lid with four ports. Gas-tight O-rings were used to connect all parts.

Three months before the start of the pre-experiment, *D. glomerata* was evenly sown (seven seeds per cosm) and established in the bottom unit of each mesocosm on top of a 12 cm thick layer of topsoil material. After one month, the leaves of each grass plant were carefully threaded through the holes of the perforated plate and left to grow in the upper unit to a mean height of 35 cm (Appendix S1: Fig. S1). To prevent gas exchange between the bottom and upper units, spare space between the grass shoots and the perforated plate was sealed with plastic paraffin film (Parafilm M, Bemis, Sheboygan Falls, Wisconsin, USA).

Four treatments were performed in two replicates each: control, $^{13}CO_2$ only, $^{13}CO_2$ + 4 grasshoppers (two female and two male), and $^{13}CO_2$ + 12 grasshoppers (six female and six male). Different numbers of grasshoppers (*C. dorsatus*) were used to create a gradient of reduced aboveground biomass. For headspace gas mixing, each cosm was equipped with a ventilator. The respective cosms were $^{13}CO_2$ pulse-labeled for two hours by installing a plastic container filled with ^{13}C-labeled sodium hydrogen carbonate ($NaH^{13}CO_3$, 99 atom%; Cambridge Isotope Laboratories, equivalent to 0.264 mmol $^{13}CO_2$/cosm) inside of each of the upper cylinders accessible via an external septum-equipped port. The cosms were then flushed with C-free air and closed gas-tight. Afterward, $^{13}CO_2$ was released by a stepwise addition of 2 mL of HCl (2 mol/L) to the $NaH^{13}CO_3$ stored in the plastic container by injection of the syringe through the septum-equipped port. The control cosms were correspondingly treated with unlabeled $NaHCO_3$.

Online gas measurements of O_2, $^{12}CO_2$, and $^{13}CO_2$ were performed using a specially designed Raman gas sensor, based on cavity-enhanced Raman spectroscopy (CERS; Keiner et al. 2014, 2015*a*, Jochum et al. 2015*a*). This sensor allows the simultaneous monitoring of multiple gases in varying concentrations between approximately 100 parts per million (ppm) and 100 Vol-%, as previously described (Jochum et al. 2015*b*, Keiner et al. 2015*b*, Sieburg et al. 2017). The gas measurement system was connected to the cosms using polyamide tubes and either flushed with C-free synthetic air in an open-circuit mode or run in a closed-circuit mode to qualify and simultaneously quantify the gas atmosphere (Appendix S1: Fig. S1). The acquisition and quantification of the gas data, and the technical conduction of the experiment (switch of magnetic valves, flushing, etc.) were automatized and controlled by a home-made software (LabVIEW, National Instruments, Austin, Texas, USA). The labeling procedure was continuously monitored using the Raman gas sensor. Thus, a precise addition of $^{13}CO_2$ was possible since the course of the gas evolution could be monitored, nonconsumptive, and online.

Results.—Immediately after the first addition of HCl to the $NaH^{13}CO_3$, the $^{13}CO_2$ level rapidly increased and then promptly declined (light blue background and lines in Appendix S1: Fig. S2a). After two hours, the labeling process was terminated and the concentration of $^{13}CO_2$ decreased continuously (Appendix S1: Fig. S2a) indicating a $^{13}CO_2$ uptake by the grass. At that point, the

gas measurement system was switched to the bottom unit. Here, concentration changes ($^{12}CO_2$ and $^{13}CO_2$ production, O_2 consumption) derived from soil respiration were monitored continuously over the next 22 h. The closed-cycle mode allowed the accumulation of CO_2 in the headspace of the bottom unit. When the CO_2 concentration reached a predefined limit (e.g., 6000 ppm), both magnetic valves were automatically opened to flush the whole system for several minutes with C-free synthetic air. After this procedure, the closed cycle was re-established by closing the magnetic valves. Gas concentrations were depicted as mixing ratios and normalized to the sum of all measured gases. The $^{13}CO_2$ and $^{12}CO_2$ evolution by grass root and microbial respiration were monitored simultaneously. The first maximum formation of $^{13}CO_2$ in the headspace was detected after 9.3 h without grasshoppers. The higher the number of grasshoppers added, the later the release of $^{13}CO_2$ in the headspace of the soil occurred with a delay of 12.2 and 13 h for four and 12 added grasshoppers (Appendix S1: Fig. S2b), respectively.

The pre-experiment exhibited a fast response of the plant–soil system to phytophagous herbivory within 24 h. As indicated by ^{13}C labeling, more grasshoppers induced a higher frass activity in concert with increased losses of leaf biomass, which resulted in diminished photosynthetically $^{13}CO_2$ uptake rates, moderated C allocation to the root system, and subsequent delayed release of $^{13}CO_2$ derived from root and microbial respiration from root exudates and rhizodepositions. Consequently, the proposed duration of five days for the key experiment with $^{13}CO_2$ pulse labeling appeared to be appropriate to induce detectable short-term effects of insect herbivory on C cycling within the plant–herbivore–soil system.

Generation of ^{15}N-labeled grasshopper feces.—Individuals of *D. glomerata* were grown for two months and then labeled by spraying with ^{15}N solution at three different abundance levels ($^{15}NH_4{}^{15}NO_3$, 1000‰, 2000‰, and 5000‰). After one day, previously caught grasshoppers (*C. dorsatus*) were added and grasshopper feces were collected over a period of eight days and analyzed for ^{15}N.

Results.—Already at an applied ^{15}N level of 1000‰, the grass biomass was sufficiently enriched in ^{15}N so that the label was passed on to the grasshopper feces. The experiment revealed first valuable information on the grass–herbivory interaction and the ^{15}N-labeled feces material of the two highest levels of ^{15}N (2000‰ and 5000‰) were kept to be applied in the key experiment. In advance, the natural $\delta^{13}C$ abundance of grasshoppers fed with *D. glomerata* and of their feces was determined from freeze-dried, ground samples.

Key herbivory experiment

The key herbivory experiment was conducted in 12 full-sized (196 L volume) mesocosms under controlled environmental conditions in a climatic chamber (15°C; automated watering; 8-h dark/16-h light cycle). The custom-made mesocosms consisted of transparent makrolon polycarbonate gaining 100 cm in height and 50 cm in diameter (Fig. 1). They consisted of a cylinder, a bottom and top lid with outlet ports. Along the cylinder, several ports were set for individual sampling of throughfall and soil solution. To collect throughfall samples, two funnels were placed randomly underneath the grass canopy. For topsoil solution sampling, one mini-glass suction cup (Ecotech, Bonn, Germany) per system was placed in 2 cm soil depth. Free draining lysimeter seepage water was collected at the outlet port of the bottom plate (Fig. 1). For headspace gas mixing, each mesocosm was equipped with two ventilators.

The same soil–gravel mixture as for the pre-experiment was used, filled (13 kg dry mass, DM) into the mesocosms to build up a 12 cm thick soil layer. Afterward, *D. glomerata* seeds were homogeneously distributed on the soil (4 seeds × 0.1 m^{-2}) by means of a punched template and left to grow. After five months, the grass was trimmed to 4 cm height to simulate grazing. No reproductive growth stage (flowering) was reached before and after clipping. One year after seeding, a dense grass cover (elongation stage) and root system were established and the five-day experiment was started. Four treatments were performed in three replicates each: C = $^{12}CO_2$ (control), L = $^{13}CO_2$, LG = $^{13}CO_2$ + 20 grasshoppers (10 female and 10 male), and LG^{15}N = $^{13}CO_2$ + 20 grasshoppers (10 female and 10 male) + ^{15}N feces. Applied grasshopper density (grasshopper biomass, 7 g/m^2)

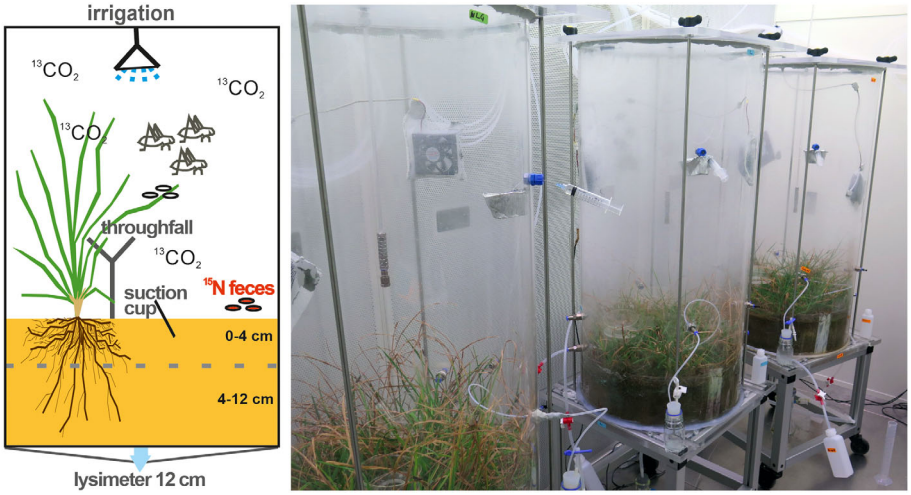

Fig. 1. Schematic mesocosm design (left) and photograph of the cosms in the climate chamber (right).

simulates a severe infestation being at the upper part of annual peak biomasses ranging between 2 and 8 g/m^2 as reported in a long-term field study by Belovsky and Slade (2000).

For the LG^{15}N treatment, 620 mg DM ^{15}N-enriched feces generated in the pre-experiment were equally distributed within a plastic ring onto the soil (95 cm^2, δ^{15}N 58‰, 6.679 mg N/mesocosm). The spatially controlled application of the ^{15}N-enriched feces became necessary for the retrieval, re-weighting, and hence to estimate the gravimetric loss of the feces material at the end of the experiment. After adding the grasshoppers (*C. dorsatus*) to treatments LG and LG^{15}N, the cosms were sealed gas-tight and flushed with C-free synthetic air. Afterward, the addition of CO_2 lasted for two hours (6.4242 mmol CO_2 for the control and $^{13}CO_2$ for the labeled cosms, +8 mL HCl stepwise application, resulting in 77.1 mg C addition per cosm).

During incubation, the mesocosms were irrigated with 2.73 L per cosm (or 13.9 mm) applying 356, 356, 1000, and 1000 mL, for days 1, 3, 4, and 5, respectively, as a mist from the top. Collected throughfall and soil solution volumes were recorded and pooled together over the five days for further analyses. Lysimeter seepage was sampled once at the end of the experiment after the last precipitation event. All collected solutions were filtered (0.45 µm cellulose acetate; Sartorius, Göttingen, Germany) and analyzed for total dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) concentrations by thermal oxidation (TOC-V_{CPN}/TNM-1; Shimadzu, Duisburg, Germany). The total volume of irrigation subtracted by the respective collected volume for sample analyses was multiplied by element concentrations to calculate input–output budgets per cosm.

After incubation, the aboveground biomass was cut down to the ground, grasshoppers freshly excreted feces, and added ^{15}N-enriched feces were sorted out, freeze-dried, and weighed. Three soil cores per cosm were taken by steel cylinders (12 cm in height, 9.3 cm in diameter) and divided into two soil depths (0–4 and 4–12 cm). The three soil samples per depth and cosm were pooled, and the root biomass was manually collected, freeze-dried, and weighed. Fresh soil material was used for the determination of microbial biomass C and N. Remaining soil was oven-dried at 40°C.

δ^{13}C values in solution samples.—The determination of δ^{13}C values in throughfall and soil solutions was performed according to Malik and Gleixner (2013). After sample filtration (<0.45 µm), the inorganic carbon was removed from solutions by acidification with phosphoric acid (8.5%). Twenty microliter H_3PO_4 was added to 1 mL sample solution, vortexed, and purged

with N_2 (99.99%) for 10 min. Afterward, samples were analyzed by LC-IRMS (HPLC mode; Thermo Fisher Scientific, Erlangen, Germany).

Soil microbial biomass.—For the determination of soil microbial biomass, the chloroform fumigation extraction analysis described by Vance et al. (1987) was performed with slight modifications according to Malik et al. (2013). Seven gram each of fresh mineral soil was weighed into Falcon tubes in triplicate for non-fumigated and in beakers for fumigated samples, respectively. The latter were set under chloroform atmosphere for 24 h. K_2SO_4 solution (0.05 mol/L, 1:4 w/v) was added to fumigated and non-fumigated samples, homogenized on a horizontal shaker (30 min), and centrifuged (5 min, 12,000 g). The supernatant was filtered twice (Whatman No. 1 and 0.45 μm), and filtrates were analyzed for total dissolved organic C (vario TOC cube; Elementar, Langenselbold, Germany) and N (TN-100; Mitsubishi, Yamato, Kanagawa, Japan). For calculation of microbial biomass C and N (MBC and MBN) amounts, the following conversion factors were used: $k_{EC} = 0.45$ (Joergensen 1996) and $k_{EN} = 0.54$ (Joergensen and Mueller 1996). According to Malik et al. (2013), a mass balance equation was used to calculate $\delta^{13}C$ values of MBC.

$\delta^{13}C$ and $\delta^{15}N$ values in solid samples.—Aliquots of each dried solid sample (grass leaves and roots, grasshoppers, feces, mineral soil) were ground and measured for organic C and total N using an elemental analyzer (vario EL cube; Elementar) and for $\delta^{13}C$ and $\delta^{15}N$ using an EA-IRMS (CE 1100 coupled via Con Flo III with a Delta+; Thermo Fisher). Delta ^{13}C values were calculated relative to the VPDB (Vienna Peedee Belemnite, $R(^{13}C/^{12}C)_{VPDB} = 0.0111802$) standard.

The total amount of ^{13}C derived from added $^{13}CO_2$–C in different compartments was estimated according to the following equation after converting the δ notation in atom% ^{13}C:

$$^{13}C_{comp}(mg) = (TOC_{comp} \times atom\%^{13}C_{comp}) - (TOC_{comp} \times atom\%^{13}C_{comp\,control})$$

where TOC_{comp} and $atom\%^{13}C_{comp}$ are the total organic carbon and ^{13}C amounts of the individual compartment per cosm, respectively. $atom\%^{13}C_{comp\ control}$ is the corresponding mean of the total ^{13}C amount of the respective compartment of the control treatments (C). For calculations, the natural $\delta^{13}C$ abundance of grasshoppers (fed by *D. glomerata*) and their feces of −28.00‰ and −32.99‰, respectively, was used.

The proportion of feces-derived N from ^{15}N-labeled feces in different compartments was estimated according to:

$$\text{Feces derived}^{15}N(\%) = \frac{((TN_{comp} \times atom\%^{15}N_{comp}) - (TN_{comp} \times atom\%^{15}N_{comp\,control}))}{(TN_{feces\,added} \times atom\%^{15}N_{feces\,added})} \times 100$$

where TN_{comp} and $atom\%^{15}N_{comp}$ are the total N and ^{15}N amounts of the individual compartment per cosm, respectively. $Atom\%^{15}N_{comp\ control}$ and $atom\%^{15}N_{feces\ added}$ are the corresponding mean of the total ^{15}N amount of the respective compartments of the ^{13}C label only treatments (L) and of the ^{15}N-labeled feces added at the beginning of the experiment, respectively. $TN_{feces\ added}$ is the total N amount of the ^{15}N-labeled feces per cosm.

Statistics

All response variables were described by mean and standard error or standard deviation as derived from the statistical models. Data were checked for normality and homogeneity of model residues, and, if necessary, were transformed using log10. Effects were analyzed with linear mixed-effects models (LMMs; Pinheiro and Bates 2000, Pinheiro et al. 2013) in the R statistical environment, R 3.5.0 (R Core Team 2018), using contributed package nlme (Pinheiro et al. 2013).

As fixed factor, we included treatment and type. We considered all respective treatments (C, L, LG, LG^{15}N) as treatment levels. Depending on the tested ecosystem compartments, type was specified for biomass compartments, for solution type, and for soil, respectively. Biomass compartments were separated into aboveground and belowground with roots collected from 0–4 and 4–12 cm soil depth interval. Solution type was separated into throughfall, suction cups, and lysimeters. Soil was separated into 0–4 and 4–12 cm soil depth.

Mesocosm ID was set as random effect in order to account for nested sampling of several biomass compartments, solution types, and soil strata within the same cosm. In order to allow for different variances for each level of type and hence to account for heteroscedasticity of the model, we finally used the varIdent class of the varFunc argument (Pinheiro and Bates 2000). The concentrations, isotopic signatures, and stocks of elements in biomass, solution, soil, and soil microbial biomass were included as response variable in the LMMs, respectively.

The final LMMs included two-way interactions of treatment and type. These full models were used for model parameter estimation unless 2-way interactions were significant or not. We applied this full model approach since full models best reflect the data structure and are less prone to alpha errors (Schielzeth and Forstmeier 2009). The final model includes variance heterogeneity among treatments or types, depending on the performance of the model in terms of the Akaike information criteria (AIC) and patterns in model residuals. During model building process, model parameters were estimated by maximum-likelihood estimation in order to compare the several models for the same response variable but with different assumptions on the random effects (Pinheiro and Bates 2000, Zuur et al. 2009). The effect of improvement of random-effects structure was tested based on residual plots, and on AIC (Akaike 1998) with the lower the value, the more information is included within the model (Zuur et al. 2009). The final model estimates were reported in the supplement (Appendix S1: Table S1) and are based on restricted maximum-likelihood estimates. Post hoc tests were performed using lsmeans/emmeans package in R, and estimates were adjusted for false discovery rate (Verhoeven et al. 2005).

Results

Solution chemistry

The impact of herbivory on solution chemistry was significantly different for aboveground (throughfall) and belowground solutions (suction and lysimeter seepage; $P = 0.026$). Compared to the control, throughfall (TF) solutions of the grasshopper treatments showed significantly higher TDN concentrations (LG, $P < 0.01$, Table 1) together with significantly lower DOC: TDN ratios (LG/LG^{15}N, $P < 0.05$, Table 1). However, no herbivory effect was notable for both soil solution types. The total N input via TF solution was on average 4- to 10-fold higher compared with the non-herbivory treatments (LG/LG^{15}N > L, $P < 0.02$, Table 1).

The concentrations of DOC differed significantly among TF and soil solutions ($P < 0.0001$) exhibiting significantly higher DOC values in soil solutions than in TF (Table 1). Treatment effects on TF DOC concentrations were found for LG compared with both control treatments (LG > C, $P = 0.048$; LG > L, $P = 0.012$) but not for soil solutions.

The ^{13}C labeling was solution-specific ($P = 0.0003$). Treatments labeled with ^{13}C exhibited enriched δ^{13}C values in TF and lysimeter solutions, while solutions from suction cups were generally less enriched (Fig. 2).

Highest ^{13}C enrichments were found in TF solutions of the herbivory treatments (Table 1, Fig. 2) being significantly higher compared with the control (C < LG, $P = 0.0083$; C < LG^{15}N, $P = 0.0054$) and by trend compared with the ^{13}C label only L treatment ($P = 0.06$).

Above- and belowground plant biomass, grasshopper, and feces

Insect herbivory reduced the aboveground biomass (DM) by 34% on average (mean difference between C/L and LG/LG^{15}N), while root biomass C remained unaffected (Table 2). The mean weight of newly excreted feces in the herbivory treatments amounted to 0.45 ± 0.11 g DM/cosm, while the mean weight (0.62 g DM) of the initially added ^{15}N-labeled feces was reduced by 80%. Herbivory had no effect on concentrations of total organic C and total N in above- and belowground biomass (Table 2). Root N concentrations in the two soil depths were significantly different for C ($P < 0.0078$) and L ($P = 0.0113$) but not among the herbivory treatments.

The ^{13}C labeling induced a significant ^{13}C enrichment ($P < 0.001$) in leaf and root biomass

Table 1. Concentrations of dissolved organic C (DOC) and total dissolved N (TDN), total amount of recovered ^{13}C, and accumulated fluxes of total N per mesocosm (via throughfall, suction cup, lysimeter) depending on treatments with C = control, L = $^{13}CO_2$ label only, LG = $^{13}CO_2$ + hopper, and LG^{15}N = $^{13}CO_2$ + hopper + ^{15}N feces.

Solution type	Treatment	DOC (mg/L)	TDN (mg/L)	DOC:TDN	Enriched ^{13}C (μg/cosm)	Total N (mg/cosm)
Throughfall	C	2.4 ± 0.6^{a}	0.3 ± 0.1^{a}	12.6 ± 3.1^{a}		0.60 ± 0.32^{a}
	L	2.3 ± 0.5^{a}	0.2 ± 0.0^{a}	15.2 ± 0.5^{a}	0.16 ± 0.14^{a}	0.37 ± 0.08^{a}
	LG	6.8 ± 0.4^{b}	1.7 ± 0.3^{b}	4.1 ± 0.8^{b}	3.41 ± 0.61^{b}	3.80 ± 0.58^{c}
	LG^{15}N	4.7 ± 0.5^{c}	1.0 ± 0.2^{ab}	5.2 ± 0.6^{b}	3.54 ± 0.92^{b}	2.23 ± 0.46^{b}
Suction cup	C	11.3 ± 2.1	1.0 ± 0.1	11.6 ± 1.1		2.54 ± 0.24
	L	11.2 ± 0.6	1.0 ± 0.1	10.8 ± 0.3	0.41*	2.20 ± 0.26
	LG	8.7 ± 1.3	1.0 ± 0.1	10.4 ± 1.7	0.77 ± 0.11	2.55 ± 0.21
	LG^{15}N	11.4 ± 1.3	1.2 ± 0.0	9.5 ± 1.4	0.65 ± 0.18	2.75 ± 0.04
Lysimeter 12 cm	C	24.5 ± 1.2	1.8 ± 0.1	13.7 ± 0.6		0.90 ± 0.10
	L	30.6 ± 1.6	2.3 ± 0.1	13.3 ± 0.6	1.55 ± 0.45	0.72 ± 0.27
	LG	24.6 ± 2.1	1.9 ± 0.1	13.2 ± 0.7	2.17 ± 0.83	0.78 ± 0.30
	LG^{15}N	29.3 ± 3.8	2.4 ± 0.4	12.1 ± 0.6	1.56 ± 0.34	0.54 ± 0.03

Note: $n = 3$, mean ± standard error; different letters signify significant differences in LMEM.
* $P < 0.05$; only one value determined.

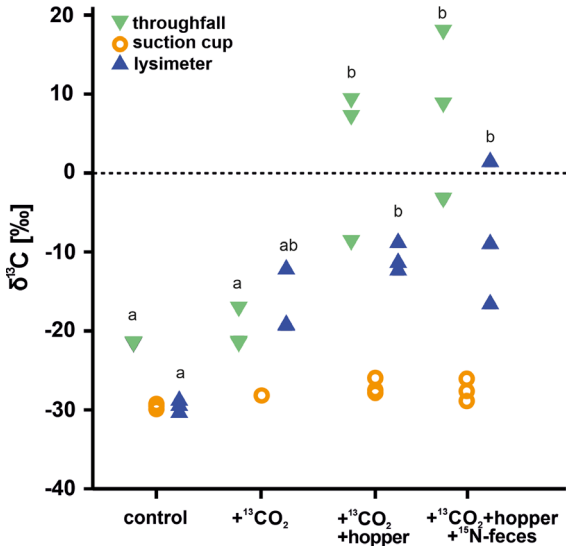

Fig. 2. $\delta^{13}C$ abundance of different solution types (throughfall, suction cup, lysimeter) per mesocosm depending on treatments. Significant differences within solution types signified by different letters (LMEM, $P < 0.05$).

(in both soil depths; Fig. 3) showing higher amounts of ^{13}C incorporation in leaves than in roots in the L treatment (Table 2). The variance of $\delta^{13}C$ values increased in all compartments (LMEM; Appendix S1: Table S1), but no herbivory effect was observed.

By trend, a treatment effect of $\delta^{15}N$ values in leaf and root biomass was observed ($P = 0.0699$; Fig. 3). The difference among compartments within one treatment significantly depended on the effect of treatment ($P = 0.0377$). There is no difference in $\delta^{15}N$ values between aboveground biomass ($P > 0.3938$) and roots in 4–12 cm soil depth ($P > 0.2572$), but significantly higher $\delta^{15}N$ values in roots of 0–4 cm depth became notable between LG^{15}N and the other ^{13}C-labeled treatments (LG^{15}N > LG, $P = 0.0452$; LG^{15}N > L, $P = 0.0173$) and by trend between the control treatments (LG^{15}N > C, $P = 0.0664$). Compared to plant biomass, freshly excreted feces of LG^{15}N treatment was clearly enriched in ^{15}N (Kruskal–Wallis test: $\chi^2 = 3.857$, $P = 0.0495$; Fig. 3).

Soil organic C and N and soil microbial biomass

The mean concentrations of organic carbon (OC) in both soil depths ranged between 1.3% and 1.7% and between 0.13% and 0.16% for total N (TN), with slightly lower C:N ratios in 4–12 cm soil depth (Table 3). The ^{15}N-labeled feces did not impact the ^{15}N signature in soil organic matter (SOM) ranging from 3.29‰ to 3.68‰ between treatments (Table 3).

In 0–12 cm soil depth, total amounts of microbial biomass C and N (MBC and MBN) ranged between 333 and 590 μg/g soil and 41–63 μg/g soil, respectively (Table 3). No herbivory effects

Table 2. Total amounts of organic C and total N, and of enriched ^{13}C in different biomass compartments (leaves, grasshoppers, feces, roots) per mesocosm depending on treatments with C = control L = $^{13}CO_2$ label only, LG = $^{13}CO_2$ + hopper, and LG^{15}N = $^{13}CO_2$ + hopper + ^{15}N feces.

Compartment	Treatment	Total OC (g/cosm)	Total N (g/cosm)	Enriched ^{13}C (mg/cosm)
Leaves	C	11.6 ± 1.7	0.36 ± 0.07	
	L	14.8 ± 1.7	0.50 ± 0.10	38.4 ± 4.7
	LG	12.5 ± 3.3	0.36 ± 0.08	25.5 ± 7.6
	LG^{15}N	13.0 ± 1.1	0.53 ± 0.08	30.6 ± 5.1
Grasshoppers	LG	0.65 ± 0.03	0.15 ± 0.01	0.51 ± 0.02
	LG^{15}N	0.65 ± 0.13	0.15 ± 0.03	0.44 ± 0.20
Feces	LG	0.23 ± 0.08	0.01 ± 0.00	0.23 ± 0.05
	LG^{15}N	0.14 ± 0.04	0.01 ± 0.00	0.17 ± 0.07
Roots 0–4 cm	C	22.7 ± 4.7	0.39 ± 0.09	
	L	16.6 ± 2.8	0.30 ± 0.07	11.3 ± 2.1
	LG	24.9 ± 8.1	0.48 ± 0.17	19.4 ± 5.3
	LG^{15}N	23.2 ± 5.6	0.43 ± 0.14	22.8 ± 5.6
Roots 4–12 cm	C	5.0 ± 0.6	0.09 ± 0.01	
	L	7.1 ± 0.7	0.14 ± 0.02	6.5 ± 2.6
	LG	4.5 ± 1.0	0.08 ± 0.02	5.2 ± 2.8
	LG^{15}N	5.7 ± 1.4	0.10 ± 0.02	6.9 ± 2.5

Note: $n = 3$, mean ± standard error.

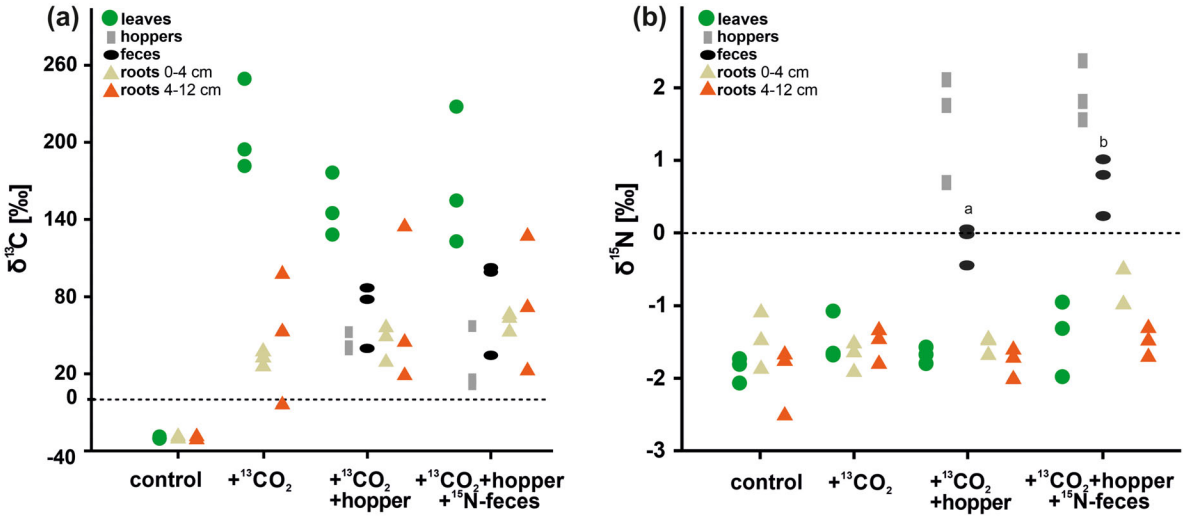

Fig. 3. (a) $\delta^{13}C$ values and (b) $\delta^{15}N$ values in different biomass compartments (leaves, grasshoppers, feces, and roots; 0–4 and 4–12 cm depth) per mesocosm depending on treatments. Natural abundances of $\delta^{13}C$ values of hoppers (feed with unlabeled *Dactylis glomerata*) and their feces are 28.01 and −32.99, respectively, and are not shown for clarity. Significant differences between feces are signified by different letters (Kruskal–Wallis, $\chi^2 = 3.857$, $P = 0.0495$).

on MBC and MBN were observed. In general, significantly higher MBC concentrations were found in 0–4 than in 4–12 cm soil depth (Table 3), while for MBN higher concentrations in the topsoil were only reported for non-herbivory treatments (C, $P = 0.0091$; L, $P = 0.0139$). MBC was only significantly enriched in ^{13}C in the L treatment compared with control ($P < 0.01$). No

Table 3. Total dry mass (DM), organic C and N amounts, $\delta^{15}N$ values, amounts of microbial biomass C and N, $\delta^{13}C$ values of microbial biomass C, and total amount of recovered ^{13}C of mineral soil samples in two soil depths (0–4 and 4–12 cm) depending on treatments with C = control, L = $^{13}CO_2$ label only, LG = $^{13}CO_2$ + hopper, and LG^{15}N = $^{13}CO_2$ + hopper + ^{15}N feces.

Mineral soil	DM (kg)	OC (%)	TN (%)	C:N ratio	$\delta^{15}N$ (‰)	Enriched ^{13}C (mg/cosm)	MBC (μg/g)	$\delta MB^{13}C$ (‰)	$MB^{13}C$ (mg/cosm)	MBN (μg/g)
0–4 cm										
C	3.9 ± 0.2	1.40 ± 0.08	0.14 ± 0.006	10.1 ± 0.2	3.50 ± 0.08		505 ± 41	−28.1 ± 0.2		56 ± 2.0
L	3.5 ± 0.1	1.54 ± 0.04	0.15 ± 0.004	10.0 ± 0.2	3.42 ± 0.04	2.2 ± 0.3	480 ± 31	32.3 ± 11.0	1.1 ± 0.3	53 ± 1.2
LG	3.8 ± 0.2	1.49 ± 0.07	0.15 ± 0.008	10.3 ± 0.2	3.65 ± 0.05	3.0 ± 1.1	651 ± 117	−0.7 ± 15.3	0.8 ± 0.2	58 ± 3.6
LG^{15}N	3.5 ± 0.2	1.51 ± 0.03	0.15 ± 0.002	10.3 ± 0.1	3.49 ± 0.02	5.5 ± 1.2	514 ± 54	33.2 ± 22.1	1.5 ± 0.7	55 ± 4.2
4–12 cm										
C	7.3 ± 0.6	1.30 ± 0.04	0.13 ± 0.004	9.6 ± 0.1	3.52 ± 0.01		397 ± 14	n.d.		46 ± 2.4
L	6.9 ± 0.5	1.31 ± 0.05	0.14 ± 0.007	9.5 ± 0.1	3.68 ± 0.06	2.4 ± 0.2	400 ± 15	−15.3 ± 12.4	2.0 ± 0.3	48 ± 1.3
LG	7.2 ± 0.3	1.66 ± 0.13	0.16 ± 0.004	10.2 ± 0.5	3.29 ± 0.19	13.1 ± 2.7	417 ± 31	12.6 ± 11.4	1.2 ± 0.3	47 ± 2.6
LG^{15}N	7.1 ± 0.2	1.48 ± 0.02	0.15 ± 0.003	9.7 ± 0.2	3.59 ± 0.08	2.9 ± 0.2	377 ± 43	20.1 ± 10.0	1.6 ± 0.3	50 ± 3.5

Note: $n = 3$; mean ± standard error; n.d., not determined.

herbivory effect was observed. The herbivory treatments exhibited a high variability of ^{13}C abundances and, by trend, a lower ^{13}C incorporation into microbial biomass in 4–12 cm soil depth (Table 3).

C and N budgets and recovery of added ^{13}C and ^{15}N

The input and output budgets of N and the C:N ratios in different compartments were averaged across the two control treatments (C + L) and individually illustrated for LG and LG^{15}N in Fig. 4. The five-day cumulative mean DOC and TDN inputs of the control treatments via TF amounted to 5.6 ± 0.8 mg C and 0.48 ± 0.16 mg N per cosm. The difference in TF DOC and TDN input between the herbivory and control treatments was characterized by additional dissolved C and N inputs derived from feces leaching and accounted for 5.5 (LG^{15}N) to 9.3 (LG) mg C and 1.8 (LG^{15}N) to 3.3 (LG) mg N per cosm. Lowest C and N system losses with seepage water were reported for the treatment with ^{15}N-labeled feces. Here, mean DOC and TDN output losses summed up to 6.6 mg C and 0.54 mg N per cosm, while all other mean output fluxes ranged between 10.2 and 11.5 mg C and between 0.78 and 0.85 mg N per cosm.

In general, the $^{13}CO_2$ treatment created enriched ^{13}C signals in all compartments (Tables 2, 3). Highest total ^{13}C amounts were found in the aboveground biomass of the $^{13}CO_2$-labeled treatment L (Table 3). In contrast, herbivory induced an enrichment of the total amounts of ^{13}C in the SOM pool and in the total belowground biomass (Table 3). ^{13}C amounts in SOM decreased in the order of 16.1 (±7.7), 8.4 (±0.9), and 4.6 (±0.6) mg ^{13}C/cosm for LG^{15}N, LG, and L, respectively. The difference between the total mean SO^{13}C amount of the L treatment and the herbivory treatments revealed a 3.8–11.5 mg increase (+83% to +250%) in accumulated ^{13}C amounts (Table 3). In the herbivory treatments, the mean additional input of ^{13}C via feces leachates was negligible (<0.003 mg ^{13}C; Table 1). An overview of the percentage of recovered ^{13}C in the respective compartments is given in Appendix S1: Table S2.

The remaining amount of ^{15}N-labeled feces comprised on average 1.59 mg N, while 5.1 mg N was mobilized (Fig. 4). More than two third of the added feces ^{15}N were leached and translocated in the system. Referring to the initial amount of ^{15}N added, ^{15}N was on average recovered as follows: 22% in SOM, 5.6% in roots, 5.5% in leaves, and 5.3% in grasshoppers/feces. The incorporation of additional feces N into leaves and newly defecated feces was accompanied by lower mean C:N ratios in the respective compartment compared with the other treatments (Fig. 4).

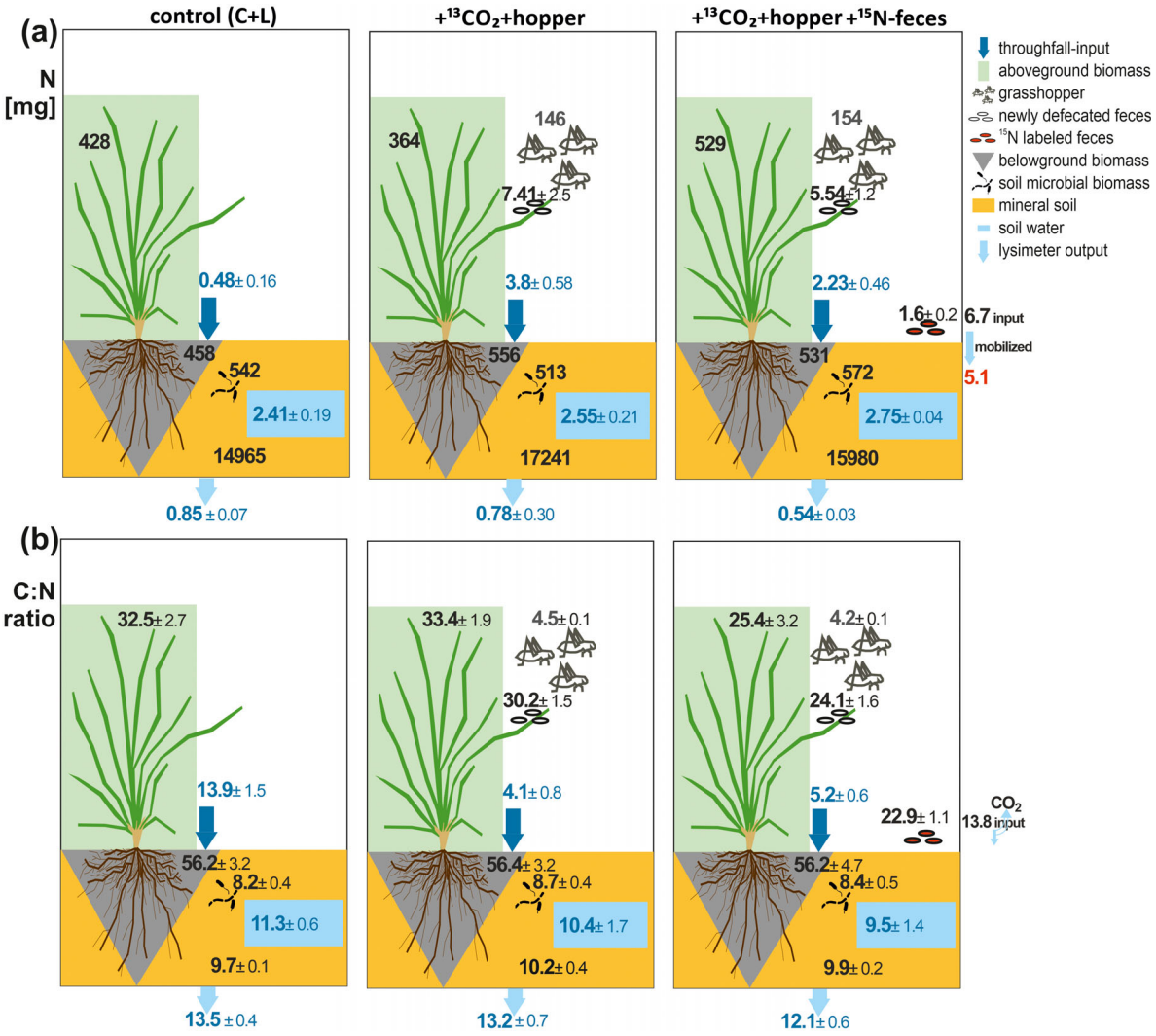

Fig. 4. (a) Mean total N amounts in solution (in blue) and in bulk compartments (in black) of the mesocosm system, and (b) corresponding C:N ratios for both control treatments (with and without $^{13}CO_2$, $n = 6$, ± standard error [SE]), for $^{13}CO_2$ + hopper ($n = 3$, ± SE), and for $^{13}CO_2$ + hopper + ^{15}N feces ($n = 3$, ± SE).

Discussion

The present study was designed to gain a better understanding of the impacts of short-term herbivory on nutrient dynamics under N-limited conditions. Our results support other findings, where besides root and microbial respiration rates (Gavrichkova and Kuzyakov 2017), the storage of labile C reserves in roots, and root exudation activity was documented to be significantly changed in the short term and in different ways after herbivore attack (Paterson et al. 2003). These specific plant–microbial interactions can lead to short-term nutrient retentions or enhanced exports depending on the biotic and abiotic properties of the ecosystem.

Plant above- and belowground interactions

In grasslands, the transport of photosynthates for the maintenance of root respiration appeared to be reduced instantly following leaf clipping (Craine et al. 1999, Wan and Luo 2003, Zhou et al. 2007). During the initial 24 h, the herbivore treatments of our pre-experiment also showed

reduced root and microbial activities as indicated by a prolonged time period (+three hours) to reach the maximum soil $^{13}CO_2$ peak compared with the control. This is in line with Gavrichkova and Kuzyakov (2017) who concluded that root and microbial respiration respond rapidly to a reduction in photosynthesis due to short above–belowground feedback loops. In a pulse labeling experiment with grasshopper herbivory on maize (24-d-old plants), Holland et al. (1996) did not find retarded response times in peaking $^{14}CO_2$ evolution. However, this might refer to the fact that labeling was applied two days after the herbivore attack had started and not simultaneously.

Previous studies revealed that recently fixed C in aboveground plant parts that survived herbivory was transported in accelerated rates to the roots (Dyer et al. 1991, Schwachtje et al. 2006, Orians et al. 2011). In this context, Schwachtje et al. (2006) reported a 10% increase in root C allocation rates in form of sugars within six hours after insect attack on wild tobacco (*Nicotiana attenuata*) using ^{11}C photosynthate labeling. These findings corroborate the fact that herbivory induced a stronger ^{13}C enrichment in roots, while shoots were less ^{13}C-enriched. This plant adaptation mechanism can be considered as a defense response to diminished accessibilities of recently fixed C following herbivory and the use of temporarily root-stored labile C reserves to facilitate plants' regrowth (Dyer et al. 1991, Holland et al. 1996, Schwachtje et al. 2006). According to the review by Orians et al. (2011), this plant response is termed "induced resource sequestration," a strategy that is especially favored by perennial, mature plants with low constitutive storage organs, which are in a phenological stage prior to seed production, and subjected to high light and low nutrient availability, as the grass plants in our experiment. Regarding the production of plant defense compounds, increased levels of secondary plant metabolites in grasshopper frass-damaged *D. glomerata* leaves were not detected as exhibited by accompanying analyses of a side experiment (Crecelius et al. 2017).

Plant root–soil microbial interactions

As an important belowground plant–soil microbial linkage (Bardgett and Wardle 2010), root exudates drive interactions between microbes in the rhizosphere and plants (Bardgett et al. 1998, Kuzyakov and Domanski 2000). Defoliation of grasses significantly impacted rhizodeposition leading to higher amounts of organic C exudations under low N availability (Paterson and Sim 2000). Moreover, transient systemic responses of plant root activity to tissue wounding were reported in previous studies (Dyer and Bokhari 1976, Hamilton III and Frank 2001, Paterson et al. 2003, 2005) exhibiting a rapid increase in root exudation of herbs and grasses over a period of two days followed by a subsequent decline to initial levels after 3–8 d. The higher incorporation of freshly assimilated ^{13}C into the SOM pool of the herbivory treatments (83–250% more ^{13}C stored) strongly supplies evidence for accelerated root exudation rates under low soil N availability since the potentially available proportion of ^{13}C from feces leachates was negligible over the course of the five-day experiment. Generally, root exudates were found to be independent from suppressed rates of photosynthate supply to the root system directly following defoliation (Paterson et al. 2003), as discussed above. The contribution of pre-defoliated carbohydrate and protein reserves to root exudation of the grass plant was found to be significantly higher within two days after defoliation (Paterson et al. 2005). This process might be one reason for the observed highly variable microbial $\delta^{13}C$ values after assumed exudate incorporation.

The competitive capacity of *D. glomerata* to acquire resources was found to be high (Poorter and Remkes 1990, Tischer et al. 2019), and microbes may have used the rapidly available substrates from root exudates and feces leachate for energy metabolism to mine nutrients from the more recalcitrant SOM pool (Kuzyakov 2010). As a consequence, a higher proportion of microbial-derived CO_2 efflux from the soil between the second day and the fifth day due to accelerated microbial activity appears possible. Furthermore, since no significant differences in soil solution N between treatments were found, a rapid net N immobilization by microbial and/or plant N uptake was assumed masking any release of N following herbivory. Plants under nutrient deficiency and hence subjected to low available N sources in the mineral soil (0.14% N) and in soil solutions (1 mg N/L) develop leaves

with wide C:N ratios and exude proportionately low amounts of organic N via roots after experiencing defoliation (Paterson et al. 2003). As a result, grasses compete with soil microbes for inorganic nutrients provided that climatic factors are not limiting (Frank and Groffman 2009, Fielding et al. 2013). According to Fielding et al. (2013), the immobilization of N generally occurred with a feces N content <2% corresponding to feces C:N ratios >20, as was held true for the newly defecated feces in the present study (LG 1.3–1.7% N, C:N 30) caused by low plant N levels (C:N 32.5).

Consequently, feces N was assumed to be partly immobilized by the soil microorganisms representing a very active part of the SOM pool, which was exhibited by the fact that the SOM pool was the largest sink for feces N (22%) in the present study. Immobilization of nutrients derived from feces and dead herbivores (fast cycle) in the more stable SOM pool via soil microbial biomass (Knops et al. 2002) has been confirmed by previous studies, which may lead to herbivore-induced alterations in plant species composition in the longer term (Belovsky 2000). Since the total aboveground N input (via TF) under herbivory was higher and significantly lower DOC:TDN ratios were detected, a higher proportion of inorganic N input was obvious. In this context, Nitschke et al. (2015) also reported higher concentrations of mineral nutrients (NO_3–N, PO_4–P, SO_4–S) in TF solutions affected by grasshopper feeding. However, no significant effect on the microbial biomass was detected after five days of herbivory, suggesting no additional immobilization of N into the microbial biomass in the studied system. Together with these findings, inorganic N forms for rapid plant uptake seemed to be already available in the soil via leaching, bypassing the microbial pool, supporting a high plant competitive capacity of *D. glomerata* under N-limited conditions.

Nevertheless, experimental limitations could have also mask potential impacts on soil microorganisms as they are: (1) The timing of sampling was likely too tardy for the detection of short-term responses in microbial biomass, as for instance suggested by Hamilton III and Frank (2001), who detected effects already after one but not after seven days after herbivory attack; (2) the applied CFE method is not suitable to distinguish between active and dormant microorganisms, hence underestimating the activation of dormant microbes (Blagodatskaya and Kuzyakov 2013); and (3) the microbial growth was limited due to multiple resource deficiencies triggered by the absence of other plant species and, hence, low plant diversity. Nitschke et al. (2015) reported only increased soil microbial biomass in the presence of both high grasshopper density and high plant diversity. This increase in microbial biomass was thought to be triggered by exceeding a certain threshold of nutrient quantity (provided by different qualities of plant litter input) and by a higher input of diverse carbon substrates via root exudates (as related to plant diversity).

Above–belowground fluxes

The TF input of significantly enriched ^{13}C together with up to 13 times higher DOC and TDN amounts indicated a fast cycling of freshly assimilated organic compounds from leaves via grasshopper feeding activity and feces deposition to the belowground system, although the applied total ^{13}C amount was minimal compared with system background values.

In comparison with findings by Fielding et al. (2013) where 58–69% of N was released from grasshopper feces (after four weeks), a release of 66% feces N over a far shorter time period was found in the present experiment. Since budgeting of herbivory C and N fluxes revealed no accelerated OC release and only minor changes in N output fluxes, our system seemed to respond with nutrient retention. For an N-deficient alpine grassland ecosystem, Martinsen et al. (2012) found that sheep grazing accelerated N cycling, but did not enhance the risks of additional N losses via soil leaching, which was validated by our model system.

The fact that we did not detect significantly higher N concentrations in the leaves of the herbivory treatment without ^{15}N feces does not mean that the grass plant did not take up more N than the control. As we were not able to measure aboveground biomass before the start of the experiment, already slight biomass differences may water the assumed accelerated plant N-uptake process. As confirmed by the fate of ^{15}N-labeled feces, 11% of the added N was incorporated by the grass biomass within five

days after ^{15}N feces deposition. Similar observations relating to the role of the plant biomass pool were reported by Frost and Hunter (2007), who recovered in a mesocosm experiment with oak saplings 15% of ^{15}N-enriched herbivore feces in foliage and fine root biomass. In the present study, ^{15}N was also detected in freshly defecated feces (0.08% of recovered ^{15}N feces). Enhanced decomposition rates of OM input by feces compared with grass litter (Bardgett et al. 1998) led to a rapid N translocation via soil solution, plant N uptake, and transfer to leaves after grasshopper feeding. Hence, a strongly accelerated N cycling due to changed litter quality input became apparent. Cross-contamination with labeled feces can be excluded since freshly excreted feces deposited on the marked area for ^{15}N-labeled feces was not used for ^{15}N analysis.

Conclusions

The studied grass species rapidly adapt to aboveground herbivory by altered C allocation and belowground N uptake. Our hypothesis of a reduced translocation of C assimilates to the root system and its subsequent effect on soil microorganisms was only partially confirmed. The initial plant physiological effect of a reduced soil respiration was accompanied by increased root exudation thereafter. These adaptations suggest an induced resource sequestration mechanism, which describes the provisioning of resources for plant regrowth after substantial insect herbivory under low N availability. Under resource limitations and herbivore attack, the above- and belowground C partitioning within the plant changed the source vs. sink relationship. The newly available C via root exudates was incorporated by soil microbes; however, no herbivory effect on their overall biomass or on their N incorporation was detected. Consequently, N immobilization by soil microbes was outcompeted by a rapid plant N uptake of freshly released inorganic N from feces deposition. As a consequence, the exploitative grass species *D. glomerata* promptly adapted to insect herbivory and successfully competed with soil microbes for nutrient acquisition to compensate for the reduction in aboveground biomass.

In essence, the retention of N was higher than the N export from insect-mediated deposits, which highlights the competitive N uptake by the grass plants following heavy insect herbivory as compensatory mechanism. We conclude that ecosystems characterized by low levels of N saturation are highly efficient at retaining N that becomes rapidly available in excess of insect feces and depositions. Hence, the severe short-term herbivory increased ecosystem N cycling in this test ecosystem by immediate immobilization rates and by efficiently preventing N losses via leaching. Already within a short period of time, soil fertility and plant adaptation strategies became driving forces for speeding up nutrient cycling in ecosystems subjected to severe insect herbivory.

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