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Greenhouse gas emissions and extracellular enzyme activity variability during decomposition of native versus invasive riparian tree litter

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Abstract Invasive plants alter riparian vegetation communities and shift biogeochemical processes by changing decomposition rates and the soil chemical environment created by leaf litter. It is unclear if this mechanism shifts nutrient dynamics favoring invasive dominance; riparian areas in the Southwestern USA invaded by salt cedar and Russian olive often still host mixed stands of native plants. To test the hypothesis that invasive plant success is related to altered litter inputs, microbial activity and nutrient cycling, we performed laboratory incubations examining greenhouse gas emissions and microbial extracellular enzyme activity (EEA). The responses of GHG flux and EAA were measured from decomposing translocated litter from native and invasive woody perennials between soils where they were growing. Litter decomposition from two invasive species (salt cedar and Russian olive) and two native trees (coyote willow and Fremont cottonwood) were tracked for 3 months. Soil respiration, carbon content and EEA were all more closely related to soil origin than leaf litter species. The highest decomposition rate was from willow soil. Soil nitrate at the end of the experiment was highest for soils collected under cottonwood. Nitrous oxide (N_2O) emissions were significantly greater from Russian olive litter than other species, on all soil types. Patterns observed here suggest that (1) plant influences on local soil properties over the lifetime of a plant have a greater control on decomposition processes than short-term litter input source, (2) EEA is strongly related to available C resources, and (3) the invasive shrub Russian olive may be responsible for previously undocumented large N_2O emissions in riparian systems in the USA.

Keywords Litter decomposition · Greenhouse gases · Invasive species · Extracellular enzymes · Nitrogen cycling · Riparian

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Introduction

Alien-invasive plant species are globally ubiquitous due to humans' purposeful and inadvertent altering of plant distributions to meet our nutritional, fiber, and ornamental whims. Once plant colonizers have been

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introduced, they may persist despite competition with native species evolved in those environments. As Elton (1958) articulated, this is often because alien species exhibit functional traits relevant to a significant ecosystem process; symbiotic N_2-fixers increase N import in N-limiting soils, early-senescing grasses accelerate fire return that reduces interspecific competition, and unique root morphology can allow for exploitation of novel soil resources (D'Antonio and Vitousek 1992; Humphrey and Schupp 2004; Nsikani et al. 2018).

When invasive plants successfully establish and ecosystem processes change, efforts to restore native plant species and communities are often rooted in the assumption that what came first must be best. It is nearly axiomatic among conservationists of the western United States' riparian areas that salt cedar (*Tamarix ramosissima* and hybrids; Gaskin and Schaal 2002) is a scourge due to its water profligacy (Audubon Society 2018), despite abundant evidence to the contrary, especially compared to native vegetation (van Hylckama 1970; Nagler et al. 2009; McDonald et al. 2015). For example, using a suite of 30 ecosystem traits including soil, geomorphology and vegetation physiology characters, Stromberg (1998) concluded that salt cedar is functionally equivalent to Fremont cottonwood (*Populus fremontii*) in the San Pedro riparian areas in southern Arizona.

Irrespective of water use, the expansion of salt cedar is still of concern as salt cedar leaves concentrate and excrete salts as a means to survive in high-salinity soils, which has implications for decomposition processes and nutrient cycling. Kennedy and Hobbie (2004) report that salt cedar leaf decomposition in streams was lower than ash (*Fraxinus velutina*) leaves, but faster than bulrush (*Scirpus americanus*). Other work reports that salt cedar decomposed faster within streams compared to Fremont cottonwood; however, salt cedar litter packs hosted lower species richness and abundance of stream macroinvertebrates compared to Fremont cottonwood at Wet Beaver Creek, Arizona (Bailey et al. 2001).

Russian olive (*Elaeagnus angustifolia*) is another Eurasian invader of western US riparian systems, and its establishment success is likely due to its hosting of symbiotic N_2-fixing microbes in its rhizome (DeCant 2008) and ability to establish in shade while utilizing soil moisture instead of groundwater (Reynolds and Cooper 2010). The presence of a symbiotic N_2-fixer in a community can enhance N resources for plant species (Khamzina et al. 2009). However, excess N can be a hindrance to native species restoration as many woody riparian species evolved in N-poor environments (Rice et al. 2004), and N deposition in excess of vegetation requirements is understood to reduce plant community diversity (Bobbink et al. 2010). If a given plant's litter modifies local soil microbial communities, feedbacks may exist to individual plant N nutrition via microbial activity that changes local pH or nitrification rates (Kourtev et al. 2003).

The question remains as to how easily salt cedar and Russian olive invade stands of native woody vegetation. A related question is how much invasive species change the soil microbial community, and can that knowledge be used in restoration efforts? In the absence of shading out competitors, competition between plants for water and other resources occurs belowground and can be mediated by the microbial community (Ludwig et al. 2004; Van Der Heijden et al. 2008). Microbial extracellular enzymes are important for organic matter depolymerization and cycling N and P within soils (Sinsabaugh et al. 2003). If invasive plants change local soil biogeochemistry and nutrient cycles, those changes are likely driven by differences in decomposing leaf chemistry and may be evidenced by altered enzyme activity.

We hypothesized that invasive shrub species alter the biogeochemical (i.e., C and N processing) role of soil microbial communities compared to native riparian plants via leaf litter inputs. We tested this hypothesis using a reciprocal transplant experiment that incubated invasive and native litters on soils from which the parent plants were collected. We assessed the interactions between plant material and soil with three different metrics of litter decomposition: mass loss, systematic measurements of greenhouse gas production from litter/soil combinations, and microbial extracellular enzyme activity (EEA) at the end of the experiment. We specifically predicted that invasive plant litter on soil conditioned by native plants would decompose faster than native plant litter on soils home to native plants, and accelerate N cycling, evidenced by higher gaseous N losses and higher potential rates of N-processing EAA, thus providing a microbial feedback to facilitate invasion.

Materials and methods

Study species

We sampled plant tissue and soil along the San Juan River near Bloomfield, New Mexico, USA (36.702 N, 107.978 W). We collected live leaves and petioles from the "natives" coyote willow (*Salix exigua*; hereafter "willow") and broadleaf cottonwood (*Populus fremontii*; hereafter "cottonwood"), and "invasive/aliens" salt cedar (*Tamarix* spp.) and Russian olive (*Elaeagnus angustifolia*). All of these species are early successional woody perennial shrubs/trees with distributions in the western USA typically limited to riparian areas. The study area was chosen for plant sampling because it had (by visual estimation) roughly similar landscape distribution/land cover across all four species and thus represents an opportunity to examine decomposition dynamics in a mixed native vs. invasive community. We chose to use fresh leaves as a decomposition substrate as we aimed to test differences in plant species litter type compared to the soil microenvironment, and mimic a realistic decomposition environment in the laboratory. Individual plants within a species selected for leaf collections were similar in size and approximate age. We collected fully expanded leaves from ten individuals of each species by hand, by defoliating the terminal 1 m of five individual branches per plant. We used a hand trowel to collect soil to a depth of 10 cm under the canopy (within 30 cm of the trunk) of each plant sampled.

Experimental design

We air-dried leaves for 72 h in paper sacks in a desiccating cabinet. We separated leaves and petioles from stems, then we ground all plant leaves with a coffee grinder so that microbial decomposition rates were dependent primarily on the chemical composition of the different species rather than their inherently different surface areas. To construct litter decomposition bags, we placed $\sim$ 2 g of ground plant material into empty tea bags (Nuiby Co, China). We dried bags with litter at 40 °C for 24 h and included the mass of litter bags as a record of pre-incubation mass. Bags were made of unbleached biodegradable hemp; however, we assume any bag mass loss to be uniform across litter/soil treatments as preliminary lab evaluations showed no mass loss in the empty bags pre and post drying. We chose these specific bags to reduce the possibility of chemical interference with decomposition that could confound our results.

Soils at the site are Green River sandy loams (Oxyaquic Torrifluvents). Soil texture, pH or initial SOC content did not appreciably vary within the study area between soils under invasive and native plants. We homogenized air-dried soil by passing through a 2-mm sieve and then a 250-μm screen (Andruschkewitsch et al. 2014). We assembled incubation chambers by placing $\sim$ 20 g of sieved soil into 120 ml sterile sample cups, and allowing to pre-incubate in sample cups for 24 h. Next, we placed a litter bag in the cup, and covered with another $\sim$ 20 g of soil, setting up replicate cups (n = 3) for each of the 16 combinations of litter and soil (factorial combinations of 4 plant species and their soil).

We added water to incubation soil and litter cups to restore samples to the water content in each soil at time of collection using a micro-pipette to ensure even distribution across the soil surface. We determined that value from the mass of water lost during air drying, so each sample differed slightly in the amount added. We then incubated sample cups in 900 ml glass jars with sealed lids and a small amount of water in the jar ($\sim$ 40 ml separate from the sample cup) to prevent desiccation of the samples. We measured gas concentration immediately after set-up was completed to give a time 0 measurement.

Decomposition measurements

We measured decomposition with three metrics: litter mass loss over the course of the experiment, greenhouse gas emissions [Carbon dioxide (CO_2) and nitrous oxide (N_2O)], and EEA. At the end of the experiment, litter bags were removed from soil cups, and excess soil sticking to bags was carefully removed by brushing soil away with a small clean paintbrush. Bags were dried for 24 h at 40 °C to replicate the pre-incubation procedure before taking the final mass. Mass loss was determined by the difference in mass of the litter bags + litter at the end of the incubation compared to the initial conditions.

Incubation jars were sealed with lids and incubated in the dark at 25 °C. Gas analysis was performed by venting jars for $\sim$ 5 min prior to measurements, then affixing a modified lid with quick-connect fittings

attached to sample tubing connected to the gas analyzer. Trace gas measurements were made with a Gasmet DX-4040 Fourier-transform infrared (FTIR) gas analyzer (Oy, Finland). The instrument was calibrated before measurements by zeroing the detector with ultra-high purity N_2. Gas measurements began immediately after adding water to the samples (dried, sieved soil + litter) (Time 0). During measurements, gas concentration was determined automatically via Calcmet software every 20 s over a 3–5 min interval. Jars were re-sealed following gas analysis. Fluxes were calculated with a linear model after correcting gas concentration for molar mass and evaluating the change in mass in the incubation chambers over measurement time. Measurements were taken daily between Time 0 and day 7, then weekly between day 7 and day 91. Carbon dioxide (CO_2) emissions from samples came from litter + soil, and are hereafter termed soil respiration (R_s). All gas fluxes were calculated from the molar mass changes in gases in the incubation jars over the length of measurement time, corrected by the volume of incubation chamber.

EEA was determined from soils at the end of the incubation. Following litter bag removal, 1 g of soil was removed and frozen at -20 °C until the time of enzyme assays. We assayed soils for a group of hydrolytic and oxidative enzymes involved in C, N, and P acquisition from the later stages of organic matter decomposition. Enzymes surveyed were as follows: cellulose-degrading β-glucosidase, chitin-degrading *N*-acetyl-glucosaminidase (NAG), the protease Leucine aminopeptidase (LAP), and acid phosphatase.

EEA methodology was modified from Saiya-Cork et al (2002) and McLaren et al. (2017). Soil was blended with a tissue tearor (Biospec Products, Inc, Bartlesville, Oklahoma) in pH 5 sodium acetate buffer and pipetted into 96-well plates, with eight analytical replicates per sample. Fluorescing, 4-methylumbelliferone (MUB) (β-D-glucoside, *N*-acetyl-α-D-glucosaminide and phosphate) or methylcoumarin (MC) (7-amino-4-methylcoumarin) tagged substrate were added. Assays were incubated at 20 °C for 3.5 h, with half-hourly measurements ensuring activity was measured in the linear range of the reaction. Sample fluorescence (i.e., cleaved substrate) was read with a BioTek Synergy HT microplate reader (BioTek Instruments Inc., Winooski, VT, USA) at 360 nm excitation and 460 nm emission. For each substrate, we measured the background fluorescence of soils and substrate and the quenching of MUB by soils, and used standard curves of MUB to calculate the rate of substrate hydrolyzed, hereafter EEA.

Mineral N and ^{13}C analysis

Ammonium (NH_4^+) and nitrate (NO_3^-) were determined from post-incubation soils extracted with 2 M KCl. Ammonium analysis follows Weatherburn (1967), whereas NO_3^- analysis follows Doane and Horwath (2003). After the assays were completed, absorbance spectra were determined with a Fisher Scientific accuSkan Go plate reader (Vantaa, Finland) and calculations to establish N concentration were performed via linear regression analysis based on standard concentrations added to plates.

Carbon stable isotopes (^{13}C and ^{12}C) are a useful indicator of C substrate quality. Fractionation of C during decomposition favors $^{12}CO_2$ production and enriches the microbial biomass and remaining C resources (i.e., higher ^{13}C content). We therefore conducted pre-incubation soil and leaf ^{13}C and ^{15}N analysis (Center for Stable Isotopes at the University of New Mexico; Table 1). Isotope results are presented in delta (δ) notation, which represents per mill deviations from standards:

$$\delta^a X = \left({}^aR_{\text{sample}} / {}^aR_{\text{standard}} - 1\right) \times 1000$$

where X is ^{13}C or ^{15}N and R is the ratio of ^{13}C:^{12}C or ^{15}N:^{14}N. Standards for isotope analysis were California Buckeye and peach leaf for plant tissue. Regression coefficients for accepted NIST values vs measured ^{13}C and ^{15}N were > 0.99 for both sets of standards.

At the end of the experiment, total organic carbon (TOC) and C stable isotopes were determined from 4

Table 1 Initial leaf carbon and nitrogen content and isotope composition for four species of riparian trees used in a reciprocal transplant decomposition experiment

	$\delta^{15}N$	$\delta^{13}C$	%N	%C	C:N
Cottonwood	− 2.24	− 29.52	1.84	40.56	22.06
Willow	− 1.77	− 29.79	1.56	41.82	26.73
Russian olive	− 1.28	− 25.56	3.29	44.02	13.37
Salt cedar	1.02	− 27.04	2.52	38.18	15.17

to 12 mg samples of post-incubation soils using a ThermoFisher Delta V Plus isotope ratio mass spectrometer (Vantaa, Finland). Solid samples were combusted to produce CO_2 in a Costech ECS 4010 CHNSO Elemental Analyzer which was then introduced to the mass spectrometer. Instrument standards used were IAEA-C7 and Mexican calcite (A. Campbell, New Mexico Tech).

Statistical analysis

Mineral N content (NO_3^- and NH_4^+), soil C (mass and ^{13}C), and enzyme activity were tested for homogeneity of variance via the Fligner-Killeen test. Two-way ANOVA models (testing the effects of litter type and soil origin) were employed when variances were statistically equal. In instances of heteroskedastic data, Kruskal–Wallis tests were employed to evaluate differences between groups, independently for litter and for soil origin. Analyses were conducted in the R statistical software package (R Core Team 2018).

Time series data for CO_2 and N_2O flux measured in the decomposition experiment were evaluated with a repeated measures, 2-factor ANOVA model considering soil origin and species of plant litter as main effects, an interaction term, and sample day as the repeated measure. Those analyses were also conducted in R (R Core Team 2018).

Results

Litter types differed in their C and N initial chemistry and stable isotope composition (Table 1). There were significant differences in the mass loss over the duration of the experiment between litter species (two-factor ANOVA; $F_{3,32} = 170.2$, $P < 0.001$), soil origin ($F_{3,32} = 10.70$, $P < 0.001$), and a significant interaction between soil and leaf type ($F_{3,32} = 3.19$, $P < 0.01$). Mass loss between the beginning and end of the laboratory incubations showed that irrespective of plant species, litter decomposed most completely on soils collected under willow (24–32% of original mass remained; Table 2). The greatest mass loss from a soil–litter combination was for Russian olive leaves decomposing on willow soil (Table 2). The least mass loss was also from willow litter, but decomposed on cottonwood soil (Table 2). The range in values for litter mass remaining after 90 days was greater when comparing a single litter type over the range of soils (15–19%) versus the range of values for a single soil type over the diversity of plant litters (4–8%).

Soil C

Carbon content of soils at the end of the experiment negatively correlated with $\delta^{13}C$ (Fig. 1). Willow soils were most enriched in ^{13}C relative to other soils. All soil types grouped similarly when %C was plotted against ^{13}C, again showing a dominant effect of soil over plant litter (Fig. 1).

Mineral N

Soil NH_4^+ concentration was significantly affected by soil origin, with the greatest concentrations observed for willow soils and the lowest values for Russian olive soil (Fig. 2; Kruskal–Wallis, $\chi^2 = 17.19$, $df = 3$, $P < 0.01$). No significant differences were found in soil NH_4^+ concentration when litter type was tested as a main effect (Kruskal–Wallis, $\chi^2 = 3.31$, $df = 3$, $P = 0.35$).

There was a significant effect of both litter and soil origin as main effects on soil NO_3^- concentration at the end of the incubation experiment (Fig. 3). The highest concentration of NO_3^- was measured from soils on which we incubated cottonwood litter for three of the four soil types, but the highest overall mean NO_3^- value came from cottonwood soils with Russian olive litter (litter, $F_{3,32} = 6.50$, $P < 0.01$; soil, $F_{3,32} = 161.99$, $P < 0.001$). There was a significant interaction between soil type and litter type for NO_3^- concentration ($F_{9,32} = 14.62$, $P < 0.001$).

Gas flux

There was a significant effect of litter species ($F_{3,785} = 37.34$, $P < 0.001$), soil origin ($F_{3,785} = 4.66$, $P < 0.01$), the interaction between litter and soil ($F_{9,785} = 3.01$, $P < 0.001$), and a significant effect of time ($F_{18,785} = 5.36$, $P < 0.001$) on soil respiration. Multiple comparisons via Tukey's HSD of R_s as a function of soil type revealed two significant pair-wise comparisons whereby salt cedar soil had greater R_s over the course of the incubations compared to soils collected under Russian olive and cottonwood, respectively (both $P < 0.001$; Fig. 4). Russian olive litter resulted in greater R_s over the

Table 2 Decomposition, as measured by remaining leaf litter mass from two invasive and two native riparian woody shrub species, following a 90-day laboratory incubation experiment

Soil	Litter							
	Cottonwood	SEM	Willow	SEM	Russian olive	SEM	Salt cedar	SEM
Cottonwood	0.473	0.005	0.482	0.006	0.438	0.011	0.468	0.003
Willow	0.307	0.035	0.252	0.006	0.243	0.003	0.324	0.014
Russian olive	0.421	0.012	0.396	0.006	0.398	0.008	0.449	0.007
Salt cedar	0.404	0.002	0.438	0.005	0.419	0.016	0.441	0.005

Leaf litter was decomposed on soil types collected under the same woody shrub species in a full-reciprocal experiment (all leaf litter types were decomposed on each soil type). Values are the mean proportion remaining relative to the leaf mass at the beginning of the experiment (± SEM, $n = 3$)

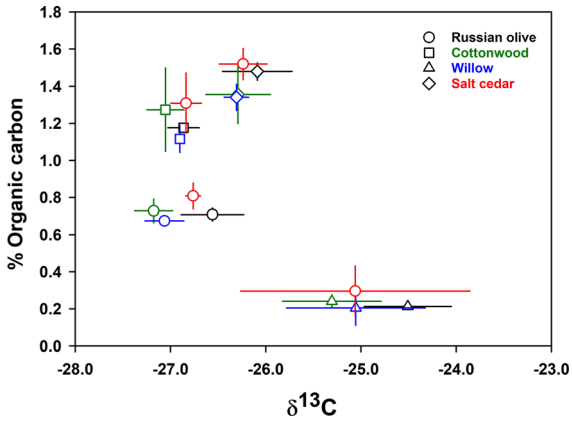


Fig. 1 Relationship between percent organic carbon (C) and the ratio of ^{13}C:^{12}C ($\delta^{13}C$) from soils following a reciprocal and soil laboratory incubation of native (cottonwood and coyote willow) and invasive (salt cedar and Russian olive) woody riparian leaf litter. Soil origin is denoted by symbols, and leaf litter decomposed during the experiment is denoted by symbol color. $\delta^{13}C$ is given as ‰. Error bars are ± 1 standard deviation from the mean

course of the incubations than any other litter species, across soil type. Soil respiration measurements did not support the hypothesis that litter decomposes faster when incubated in soils collected from under the same plant species (Fig. 4).

Nitrous oxide emissions were significantly affected by litter species ($F_{3,803} = 37.34$, $P < 0.001$), soil origin ($F_{3,803} = 4.66$, $P < 0.01$), and the interaction between litter and soil ($F_{9,803} = 3.01$, $P < 0.001$). In general, the N_2O emissions, by rank were as follows: cottonwood > Russian olive > salt cedar > willow. Among plant litters, Russian olive decomposition on any of the soil types resulted in a striking temporal pattern whereby emissions peaked after approximately 10 days for three of the soil types and after 30 days on willow soil (Fig. 5).

Soil enzyme activity

Exo-enzyme activity (EEA) was more strongly controlled by soil origin than litter additions (Fig. 6). Leucine aminopeptidase (LAP) activity was greater on cottonwood and salt cedar soils compared to willow and Russian olive soils (Kruskal–Wallis, $\chi^2 = 17.19$, $df = 3$, $P < 0.001$), but there was no difference in LAP activity among litter types ($P = 0.35$). Beta-1,4-glucosidase and phosphatase followed a similar pattern as we measured significantly higher activity of those enzymes on cottonwood and salt cedar soils compared to willow and Russian olive soils (ß-1,4-glucosidase, Kruskal–Wallis, $\chi^2 = 37.16$, $df = 3$, $P < 0.001$; phosphatase, $\chi^2 = 39.40$, $df = 3$, $P < 0.001$), but there was no effect of litter type for either enzyme (ß-1,4-glucosidase, $P = 0.18$; phosphatase, $P = 0.57$). *N*-acetyl-glucosaminidase (NAG) was the only enzyme assayed that significantly differed in activity due to both soil and litter species (2-way ANOVA, soil $F = 161.99$, $P < 0.001$; litter $F = 6.50$, $P = 0.001$).

EEAs generally showed a positive relationship with soil C content and a negative relationship with $\delta^{13}C$ (Fig. 6). Carbon dynamics clustered around soil origin rather than litter species in a similar manner as litter mass loss: we measured the lowest enzyme activity from samples containing willow litter, which also had the lowest C content by percent (0.24%), and the isotopically heaviest C (− 24.98‰; Fig. 1). For specific EEAs, NAG and acid phosphatase activity

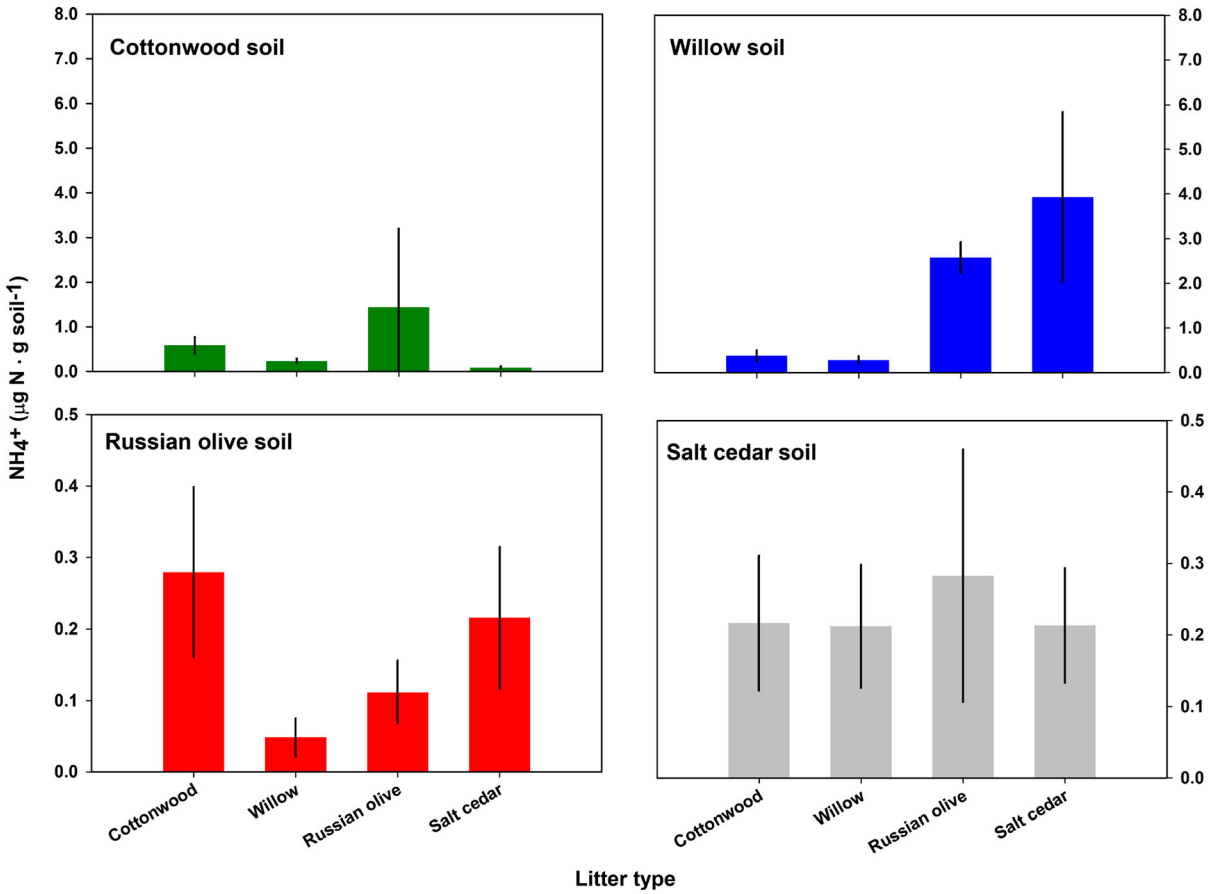


Fig. 2 Ammonium (NH_4^+) content of soils following a reciprocal and soil laboratory incubation of native (cottonwood and coyote willow) and invasive (salt cedar and Russian olive) woody riparian leaf litter. Soil origin is represented on different panels, and leaf litter decomposed during the experiment is highlighted on *x*-axis. Bar height is the mean of 4 replicates, error bars are ± 1 standard deviation from the mean

displayed the most dramatic grouping of soil type by $\delta^{13}C$, and the highest activity rates were on soils that had been collected under tamarisk, irrespective of leaf litter type (Fig. 6).

Discussion

Concern about invasive alien plant species is well justified, especially in ecologically sensitive areas like riparian zones. Potential impacts of these plant community changes are alterations to the local hydrology, allochthonous nutrient inputs, and fire regimes (Brooks et al. 2004; Predick and Turner 2008). These impacts, either observed or predicted, are the drivers of efforts in many areas to restore riparian systems to native vegetation. However, efforts to replace plant communities at the landscape or regional scale at which invasive plants have taken hold are necessarily intense, and can be economically costly. Mechanical thinning is laborious, and treatments with chemical herbicides are costly and potentially harmful to non-target organisms (Shepard et al. 2004). Therefore, determining the value of the ecosystem services that are most meaningful for successful restoration such as decomposition and nutrient cycling should enter into discussions about habitat alteration.

Discussions of invasive plant removal often solely focus on plants, and our results argue for more targeted data acquisition and monitoring on soils in invaded systems. Contrary to our expectations, soil source, rather than plant litter, had a greater effect on decomposition, soil respiration, and microbial EEA. Previous workers have shown that mixing litters of

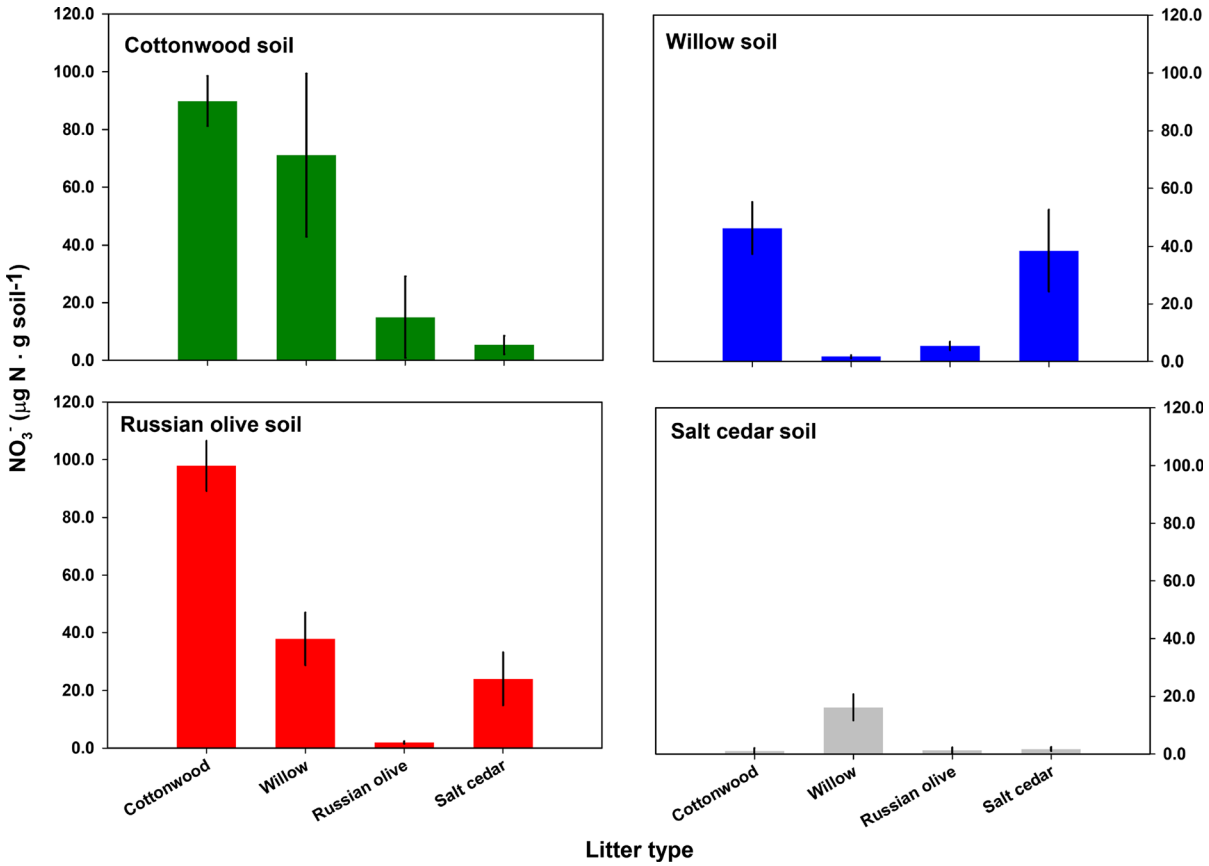


Fig. 3 Nitrate (NO_3^-) content of soils following a reciprocal and soil laboratory incubation of native (cottonwood and coyote willow) and invasive (salt cedar and Russian olive) woody riparian leaf litter. Soil origin is represented on different panels, and leaf litter decomposed during the experiment is highlighted on *x*-axis. Bar height is the mean of 4 replicates, error bars are ± 1 standard deviation from the mean

different species and functional groups has non-additive effects, i.e., ecosystem consequences of decomposition are not easily predicted from the number of species represented in a consortium of litter (Wardle et al. 1997; McLaren and Turkington 2011). In a companion study in this same riparian system, we did not observe significant differences along transects of overbank soils for physiochemical metrics like pH, electrical conductivity or moisture contents (Duval *unpublished data*). Soil properties are necessarily a function of the plants growing on them and dependent of leaf litter inputs over the life span of the plants (Rhoades 1996). However, trees affect the soil system through their uptake of soil nutrients and microbial symbionts associated with the root system. Recent work in a semi-arid system suggested that vegetation plays a more important role than soil in structuring extracellular enzymatic stoichiometry (Cui et al. 2018); however, given the latent physiochemical similarity of soils in this study, the short-term effect of our direct litter additions may not be as strong as the longer-term influence of plants shifting soil conditions through litter build-up and rhizosphere processes. In other words, plants likely dictate the soil effects observed here.

The differences in R_s observed across soils seem somewhat paradoxical given that willow litter decomposed most fully on willow soils, yet R_s from salt cedar soils was higher than other soils on most sample dates. C-hydrolyzing EEA (ß-1,4-glucosidase and NAG) were also lowest on willow soils, which appear to be related to the low concentration of organic C at the time of assays (Fig. 6). However, ^{13}C enrichment in those willow soil samples could be inferred to be a result of high respiration losses in this experimental system since we did not have to contend with isotopic

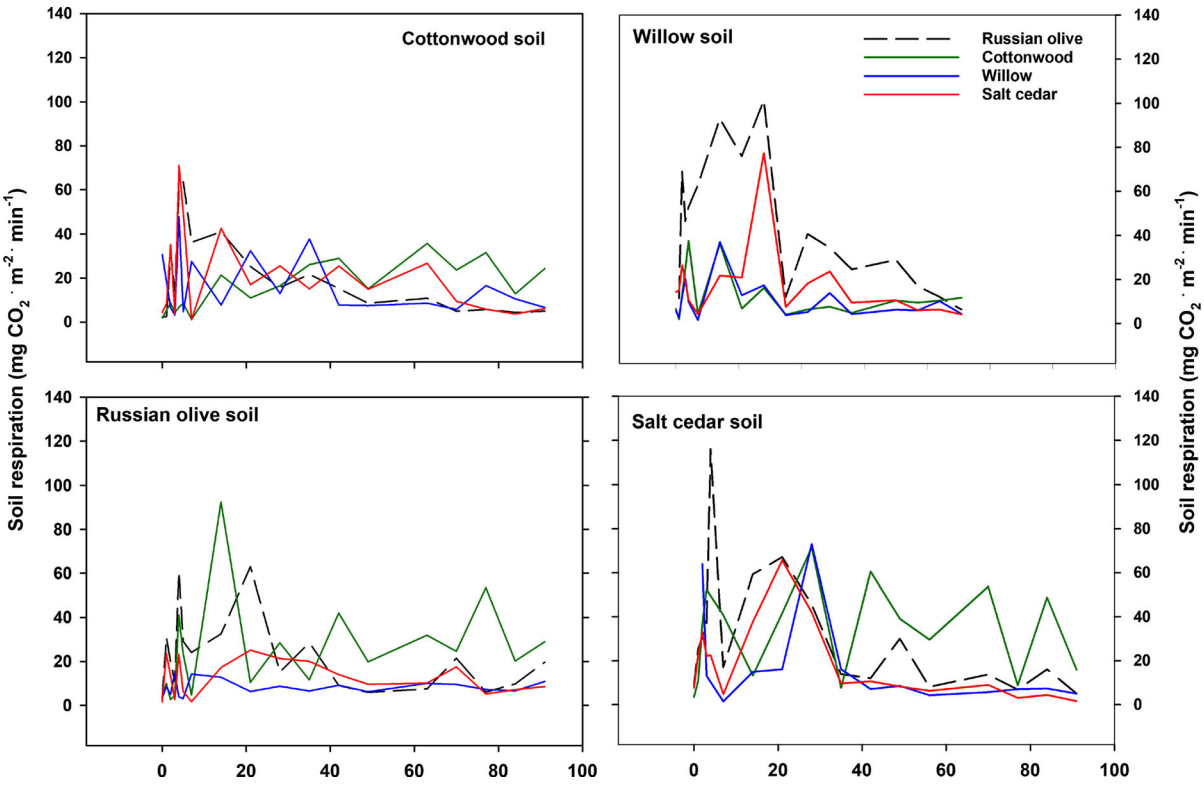


Fig. 4 Time series of soil respiration (CO_2 emissions) measured during a reciprocal and soil laboratory incubation of native (*Populus* and *Salix*) and invasive (*Tamarix* and *Elaeagnus*) woody riparian leaf litter. Soil origin is displayed in different panels, and leaf litter decomposed during the experiment is highlighted by line color. Values are mean emissions per measurement day

vagaries introduced by active plant roots and mycorrhizal fungi (Bhupinderpal-Singh 2003). When did those respiration losses occur? There is not a strong trend of high R_s at the beginning of the incubations and quickly dropping, but the loss of over 75% of the litter mass by the end of the experiment implies that the R_s signature is more strongly dominated by the soil rather than the litter. We posit that this result argues for studies of this nature to include multiple lines of evidence that point to different time scales during decomposition. Mass loss is cumulative, gas flux is a more immediate metric of microbial activity, and enzyme activity data are better thought of as a potential measurement of decomposition but one that is dependent on soil and litter properties (McLaren et al. 2017).

Even though it is well established that Russian olive hosts symbiotic N_2-fixing microbes, our end-of-incubation measurements showed that soil NH_4^+ was the lowest on Russian olive soil, likely as a result of high nitrification/denitrification rates, and concomitant N_2O from both of those pathways during the experiment. While N import may be a crucial factor in Russian olive invasiveness on N-poor soils, large gaseous N losses may preclude other species from taking advantage of those N inputs (DeCant 2008). Our data suggest that Russian olive may be accelarating riparian N cycling instead of changing N pools in a given area. Given that willow and cottonwood have likely evolved under low N conditions, physically removing Russian olive may be a viable restoration strategy if N-rich litter is not increasing soil N pools

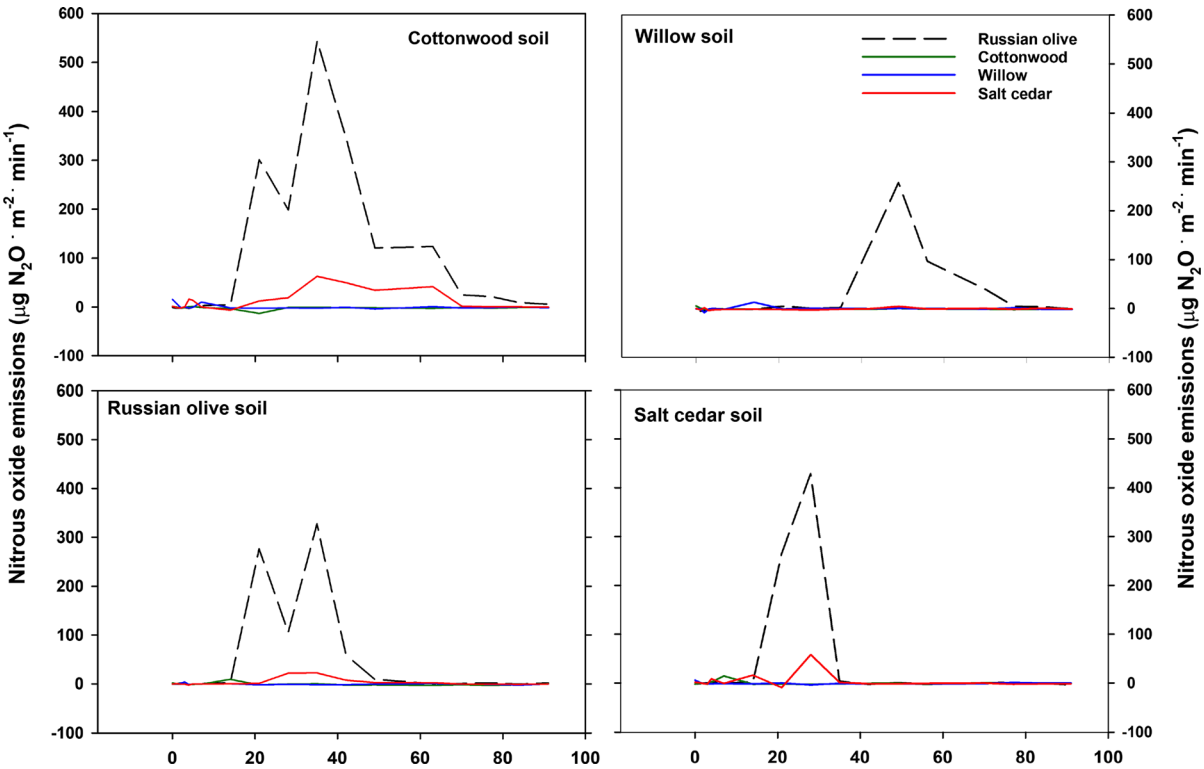
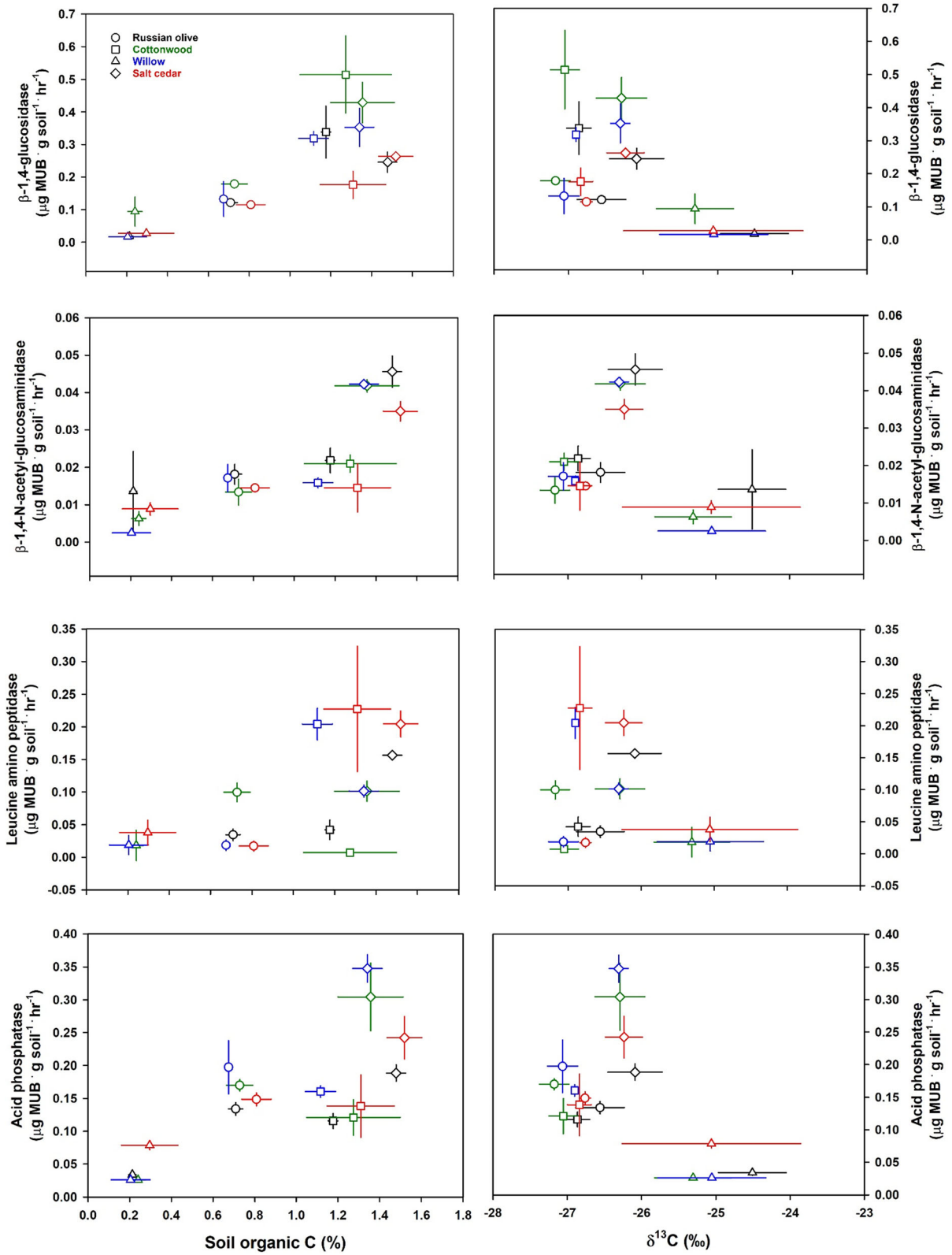


Fig. 5 Time series of nitrous oxide (N_2O) emissions measured during a reciprocal and soil laboratory incubation of native (cottonwood and coyote willow) and invasive (salt cedar and Russian olive) woody riparian leaf litter. Soil origin is displayed in different panels, and leaf litter decomposed during the experiment is highlighted by line color. Values are mean emissions per measurement day

given the high rates of N_2O production we observe relative to other plant species litters. While we did not track changes in soil N through time, the final chemical analysis showed that no litter type decomposing on its native soil (i.e., cottonwood leaves on cottonwood-derived soil) exhibited the highest amount of soil NH_4^+ (Fig. 2). However, cottonwood soil hosted the highest concentration of NO_3^- from decomposing cottonwood leaves (Fig. 3). The relatively high NO_3^- content of soils under cottonwood is worth further investigation as this property is related to N_2O emissions as well, and has been correlated with cottonwood stand age in other parts of the southwestern USA (Stromberg 1998).

While greater gaseous N losses would remove labile N from riparian soils and potentially create a more favorable environment for native tree reintroductions, it also suggests that areas invaded by Russian olive are potentially underappreciated sources of N_2O emissions to the atmosphere. Further work at the landscape and regional scale needs to address this interaction between invasive shrub ecology and greenhouse gas emissions via litter decomposition.


Russian olive
Cottonwood
Willow
Salt cedar
β-1,4-glucosidase (μg MUB · g soil⁻¹ · hr⁻¹)
β-1,4-N-acetyl-glucosaminidase (μg MUB · g soil⁻¹ · hr⁻¹)
Leucine amino peptidase (μg MUB · g soil⁻¹ · hr⁻¹)
Acid phosphatase (μg MUB · g soil⁻¹ · hr⁻¹)
Soil organic C (%)
δ13C (‰)

◀**Fig. 6** Microbial exo-enzyme activity rates in response to soil organic C (%) and $d^{13}C$ values following a 90-day laboratory incubations of invasive and native leaf litter decomposing under identical abiotic conditions on soils collected under each of the four plant species in a fully reciprocal design, i.e., all litter types were decomposed on all soil types. Soil origin is denoted by symbols, and leaf litter decomposed during the experiment is highlighted by symbol color. $\delta^{13}C$ is given as ‰. Error bars are ± 1 standard deviation from the mean

Author contributions BDD devised the experimental work, analyzed data, and wrote the manuscript. HC and AH ran lab incubation experiments, collected data and assisted with flux calculations. JM supervised laboratory work and edited the manuscript. JRM ran enzyme activity assays, assisted with data analysis, and edited the manuscript. DC performed field work, helped devise field collections and experiment design, and edited the manuscript.

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Data availability Data sufficient to re-analyze statistical models and verify results will be deposited on figshare (www.figshare.com).

Compliance with ethical standards

Conflict of interest None declared for any author.

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