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Plant litter chemistry alters the content and composition of organic carbon associated with soil mineral and aggregate fractions in invaded ecosystems

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Abstract

Through the input of disproportionate quantities of chemically distinct litter, invasive plants may potentially influence the fate of organic matter associated with soil mineral and aggregate fractions in some of the ecosystems they invade. Although context dependent, these native ecosystems subjected to prolonged invasion by exotic plants may be instrumental in distinguishing the role of plant–microbe–mineral interactions from the broader edaphic and climatic influences on the formation of soil organic matter (SOM). We hypothesized that the soils subjected to prolonged invasion by an exotic plant that input recalcitrant litter (Japanese knotweed, *Polygonum cuspidatum*) would have a greater proportion of plant-derived carbon (C) in the aggregate fractions, as compared with that in adjacent soil inhabited by native vegetation that input labile litter, whereas the soils under an invader that input labile litter (kudzu, *Pueraria lobata*) would have a greater proportion of microbial-derived C in the silt-clay fraction, as compared with that in adjacent soils that receive recalcitrant litter. At the knotweed site, the higher C content in soils under *P. cuspidatum*, compared with noninvaded soils inhabited by grasses and forbs, was limited to the macroaggregate fraction, which was abundant in plant biomarkers. The noninvaded soils at this site had a higher abundance of lignins in mineral and microaggregate fractions and suberin in the macroaggregate fraction, partly because of the greater root density of the native species, which might have had an overriding influence on the chemistry of the above-ground litter input. At the kudzu site, soils under *P. lobata* had lower C content across all size fractions at a 0–5 cm soil depth despite receiving similar amounts of *Pinus* litter. Contrary to our prediction, the noninvaded soils receiving recalcitrant *Pinus* litter had a similar abundance of plant biomarkers across both mineral and aggregate fractions, potentially because of the higher surface area of soil minerals at this site. The plant biomarkers were lower in the aggregate fractions of the *P. lobata*-invaded soils, compared with noninvaded pine stands, potentially suggesting a microbial co-metabolism of pine-derived compounds. These results highlight the complex interactions among litter chemistry, soil biota, and minerals in mediating soil C storage in unmanaged

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ecosystems; these interactions are particularly important under global changes that may alter plant species composition and hence the quantity and chemistry of litter inputs in terrestrial ecosystems.

KEYWORDS
mineral-associated carbon, plant invasion, *Polygonum cuspidatum*, *Pueraria lobata*, soil aggregates, soil organic matter

1 | INTRODUCTION

Soils store the largest reserve of organic carbon (C) in terrestrial ecosystems (Lehmann & Kleber, 2015; Post, Emanuel, Zinke, & Stangenberger, 1982; Schlesinger, 1977). The sequestration potential of C in soils is regulated by the chemical composition of plant litter that funnels atmospheric C to soils, by the physiology of soil heterotrophs that control the litter degradation and the formation of soil organic matter (SOM) through the breakdown and re-synthesis of plant inputs, and by the soil mineral matrix, which stabilizes SOM through physiochemical associations (Cotrufo, Wallenstein, Boot, Denef, & Paul, 2013; Kogel-Knabner, 2002, 2017; Schmidt et al., 2011; Six, Conant, Paul, & Paustian, 2002). The chemical association of SOM with mineral surfaces through formation of organo-mineral complexes (chemical protection; Blanco-Canqui & Lal, 2004; Kleber, Sollins, & Sutton, 2007; Six et al., 2002) and the physical occlusion of SOM within soil aggregates (physical protection; Kogel-Knabner et al., 2008) dominates the physiochemical associations that provide stability to soil C and decrease the breakdown of the SOM by soil biota (Angst, Mueller, Kogel-Knabner, Freeman, & Mueller, 2017). Although plant inputs form the precursor of a majority of the SOM (Hatton, Castanha, Torn, & Bird, 2015; Kogel-Knabner, 2002), their influence on the quantity and composition of mineral-associated and aggregate-protected pools of SOM remains less well understood (Kogel-Knabner, 2017; Prescott, 2010).

On a broad scale, climate and soil parent material influence soil C cycling by regulating both plant productivity and the development of soil profiles (Torn, Swanston, Castanha, & Trumbore, 2009). However, on a more proximate level, the formation of SOM is dynamically regulated along a plant–microbe–mineral continuum by biotic and abiotic interactions (Figure 1; Cotrufo et al., 2015; Geyer, Kyker-Snowman, Grandy, & Frey, 2016; Hatton et al., 2015; Kallenbach, Grandy, Frey, & Diefendorf, 2015; Kleber et al., 2007; Tamura & Tharayil, 2014). For example, fungal communities capable of degrading plant heteropolymers are dominant in ecosystems that receive recalcitrant plant inputs that are abundant in lignins, tannins, and cuticular matrices (Fierer, Strickland, Liptzin, Bradford, & Cleveland, 2009; Paterson et al., 2008; Voriskova, Brabcova, Cajthaml, & Baldrian, 2014). The associated slower rate of fungi-mediated litter decomposition, which generally lags behind the rate of litter input, results in the accumulation of plant heteropolymers in soils (Baldock et al., 1997; Rasse, Rumpel, & Dignac, 2005; Von Lutzow et al., 2006; Winkler, Haumaier, & Zech, 2005). Moreover, the higher proportion of residues that remain at the final stages of the decomposition of recalcitrant litter are incorporated into soils through a physical transfer pathway (Cotrufo et al., 2015) thus further increasing soil C in such ecosystems. Mycorrhizal networks, which are abundant in these polyphenol-rich, nutrient-poor ecosystems, promote soil aggregation (Rillig & Mummey, 2006) and further contribute to the physical protection of recalcitrant plant matter. In contrast, plant litter with a higher proportion of easily degradable compounds, such as carbohydrates and proteins, facilitates faster cycling of C in the soil, thus resulting in a higher production of dissolved organic matter that associates with soil minerals (Cotrufo et al., 2013, 2015). In the presence of labile C inputs, decomposers can also co-metabolize C compounds that are otherwise physically stabilized on soil minerals (Keiluweit et al., 2015), thus decreasing the overall quantity of SOM (Fontaine et al., 2007; Kuzyakov, 2010; Kuzyakov, Friedel, & Stahr, 2000). Therefore, the chemistry of plant litter influences the content and chemistry of the organic matter in different soil size fractions even within a similar edaphic and climatic envelope. Although much research has explored the effect of litter chemistry on the quantity of SOM in bulk soils (Hatton et al., 2015; Mambelli, Bird, Gleixner, Dawson, & Torn, 2011), relatively little is known about the influence of contrasting litter chemistries on the quantity and composition of C pools associated with soil mineral and aggregate fractions in natural ecosystems.

Impact of invasive plant species on soil carbon is dependent on multiple factors including the identity of the invader, the identity of native species that is being displaced, land-use pattern, soil mineralogy and climate; hence, the same invader may have contrasting influence on the SOM storage across its invaded ranges (Craig, Pearson, & Fraterrigo, 2015; Dornbush, 2014; Kramer, Warren, Tang, & Bradford, 2012; Liao et al., 2008; Martin, Newton, & Bullock, 2017). Thus, although the outcomes are highly context dependent (De Deyn, Cornelissen, & Bardgett, 2008), some of the native ecosystems subjected to prolonged invasion by non-native plants may serve as an appropriate system to partly distinguish the role of plant–microbe–mineral interactions in the formation of SOM from those of the broader edaphic and climatic influences. Exotic plants that functionally differ from the resident native species have a better chance of becoming invasive (Levine, Adler, & Yelenik, 2004; Strauss, Webb, & Salamin, 2006; Van Kleunen, Weber, & Fischer, 2010), and hence, many successful plant invaders exhibit unique biomass chemical compositions that are novel to the introduced ecosystems (Callaway et al., 2008; Macel, De Vos, Jansen, Van Der Putten, & Van

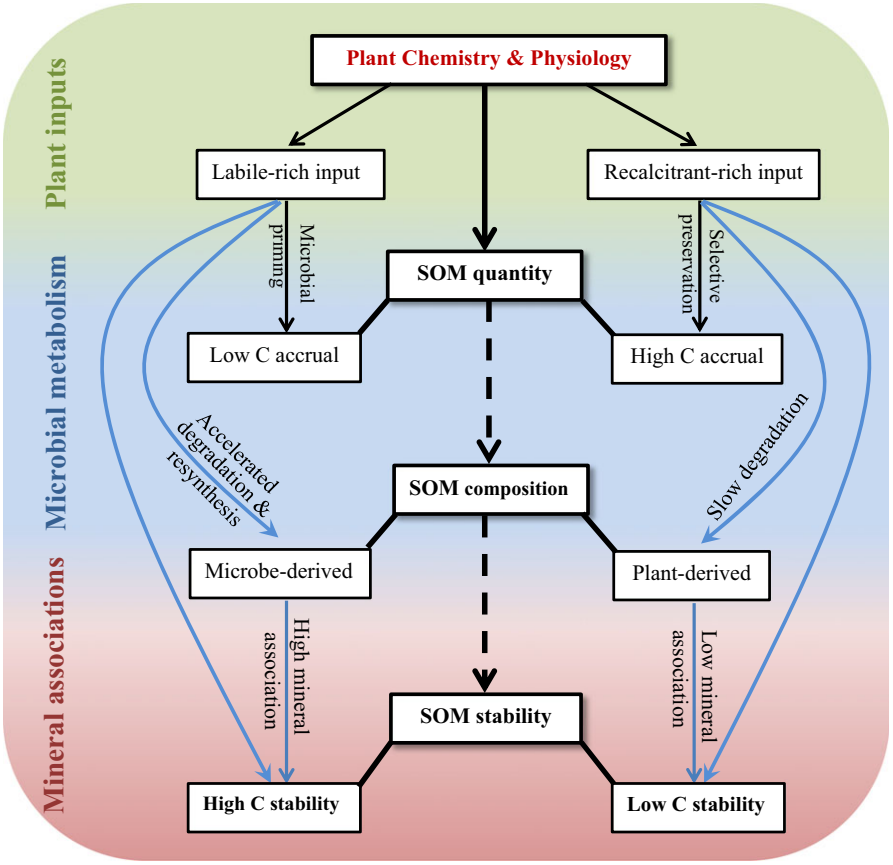

FIGURE 1 Schematic of the potential soil organic matter formation pathways operating along a plant–microbe–mineral continuum, which is mediated by plant litter chemistry (Cotrufo et al., 2015; Hatton et al., 2015), physiology of the soil heterotorphic community (Geyer et al., 2016; Kallenbach et al., 2015), and mineral associations (Keiluweit et al., 2015; Kleber et al., 2007). Blue and broken arrows represent less known mechanisms. Modified with permission from Tamura and Tharayil (2014) [Colour figure can be viewed at wileyonlinelibrary.com]

Dam, 2014; Penuelas et al., 2010; Ridenour, Vivanco, Feng, Horiuchi, & Callaway, 2008; Strauss et al., 2006). These altered plant chemistries may influence both the composition and abundance of soil heterotrophs by modulating multi-trophic interactions (Austin & Ballare, 2014; Belnap, Phillips, Sherrod, & Moldenke, 2005; Callaway, Thelen, Rodriguez, & Holben, 2004; Wardle, Yeates, Barker, & Bonner, 2006). Additionally, because of the efficient resource acquisition and use (Funk & Vitousek, 2007; Tharayil et al., 2009), many exotic plants produce higher amounts of biomass (Van Meerbeek et al., 2015) than do the native species they displace. Therefore, many invasive plants add disproportionate quantities of chemically distinct litter to their introduced ecosystems and thus may potentially influence SOM formation. Although these ecosystems experience significant perturbations during the initial years of invasion, the heterotrophic community (including soil microbes and fauna) in the invaded sites shifts over time under the selection pressure of the exotic plants, and some of these invaded ecosystems tend to reach a novel state over decadal timescales (Gaertner et al., 2014; Hobbs et al., 2006, Hobbs, Higgs, & Harris, 2009; Seastedt, Hobbs, & Suding, 2008). These novel invaded ecosystems, which coexist in proximity (usually within a few meters) to the native ecosystem and share the same climatic and edaphic envelope but have different biotic components (Kulmatiski, Beard, Stevens, & Cobbold, 2008; Peltzer, Allen, Lovett, Whitehead, & Wardle, 2010; Peltzer et al., 2009), may provide an ideal framework (Sax et al., 2007; Vitousek, 1990) to distinguish the role of plant–microbe–mineral interactions in SOM formation from those of climatic and edaphic factors.

To investigate the influence of plant invasions and the associated litter chemistry on the accrual and composition of C in the mineral-associated and aggregate-protected pools, we selected two perennial, noxious, invasive species with distinct litter chemistry: *Polygonum cuspidatum* (Japanese knotweed), which produces stem and leaf litter rich in polyphenols (Suseela, Tharayil, Xing, & Dukes, 2013; Tharayil, Alpert, Bhowmik, & Gerard, 2013; Table S1, Fig. S1a), and leguminous *Pueraria montana* var. *lobata* (kudzu), which produces nitrogen-rich labile leaf litter (N fixation—235 kg N ha^{-1} $year^{-1}$; Hickman, Wu, Mickley, & Lerdau, 2010; Lindgren, Castro, Coiner, Nurse, & Darbyshire, 2013; Table S2, Fig. S1b). The influence of litter chemistry on the formation of SOM was further emphasized by choosing study sites where these invasive species were encroaching into ecosystems with contrasting resident litter chemistries. We selected sites where *P. cuspidatum* was invading an old-field ecosystem dominated by grasses and forbs that produced relatively labile litter (*knotweed site*), whereas *P. lobata* was found to be invading a *Pinus* forest with recalcitrant-rich litter (*kudzu site*; Table S2; Tamura & Tharayil, 2014).

We hypothesized that within a same site that experience similar edaphic and climatic conditions, (i) the soils that receive litter rich in recalcitrant compounds (soils under *P. cuspidatum* and *Pinus* stands) would have a greater proportion of C in the aggregate fractions, whereas soils receiving litter rich in labile compounds (soils under old-field and *P. lobata* stands) would have a greater proportion of C in the silt-clay fraction. Due to the potential microbial co-metabolism of the plant-derived carbon, we hypothesized that (ii) compared with the soils that are adapted to the input of recalcitrant litter from native species (soils under *Pinus* stands), the adjacent soils under plant invader that inputs labile litter (soils under *P. lobata*) would have a lower C in the aggregate fraction. Further, from the perspective of SOM composition, we hypothesized that (iii) the input of recalcitrant litter would increase the plant-derived compounds in soil aggregate fractions compared with the silt-clay fraction, whereas an input of labile litter into native systems that are adapted to recalcitrant litter would decrease plant-derived compounds proportionately more from the aggregate fractions than from the silt-clay fraction.

2 | MATERIALS AND METHODS

2.1 | Study site

We selected study sites for *P. cuspidatum* in Amherst, Massachusetts (42°24′N, 72°31′W) and *Pueraria lobata* in Seneca, South Carolina (34°41′N, 82°53′W). These sites were selected as they were closer to the location of initial introduction of the respective species in the USA thus had well-established and stable invaded sites with invasion dating back to ca. 20 years (Barney, Tharayil, Ditommaso, & Bhowmik, 2006; Tharayil et al., 2013). To elucidate the effect of contrasting litter chemistries on SOM characteristics within a same edaphic and climatic envelope, study sites were selected such that at both sites the respective invasive species were encroaching into ecosystems with contrasting resident litter chemistries. At the knotweed site, *P. cuspidatum* was actively invading into an old-field that was abandoned during 1990–1992 after many years of alfalfa cultivation. At this site, *P. cuspidatum* formed monocultural stands of >500 m^2 extending from the field margins and was invading into the old-field that was dominated by grasses and forbs (*Dactylis* sp., *Elytrigia repens*, *Galium* sp., *Lepidium* spp., *Oxalis stricta*, *Plantago* spp., *Rhus glabra*, *Schedonorus phoenix*, *Setaria pumila*, *Trifolium* sp., and *Vicia* sp; Tharayil et al., 2013). At the kudzu site, *P. lobata* was invading into a pine forest dominated by *Pinus taeda* trees that were ca. 30 years of age. During the growing season, *P. cuspidatum* stands had no other understory plants, and the understory vegetation was very sparse under the *Pinus* stands.

At both sites, we randomly selected *six monospecific stands* of the invasive species. Within each invaded site, these stands served as independent experimental units ($n = 6$). Within each stand, we selected *three parallel transects* perpendicular to the advancing edge of invasion at 6–8-m intervals. Along each transect, we demarcated *one 1-m^2 sampling plot* in the *invaded zone* (~8 m inside from the edge of invasion) and one 1-m^2 sampling plot in the *noninvaded zones* (~3–4 m beyond the advancing edge of invasion). At both sites, the invaded and noninvaded plots had similar soil characteristics including soil texture, bulk density, and pH (Suseela, Alpert, Nakatsu, Armstrong, & Tharayil, 2016; Tamura & Tharayil, 2014; Tharayil et al., 2013). At the knotweed site, since our sampling in 2011, *P. cuspidatum* had encroached into >40% of the noninvaded plots, as well as the *P. cuspidatum* stands that were spatially distinct at the time of sampling has begun to coalesce, further indicating the uniformity of edaphic factors across the invaded and noninvaded plots.

In July 2011, after carefully removing the top organic layer, we collected three soil cores (10 cm diameter) of mineral soils from each plot to a depth of 15 cm at 5-cm intervals. The soils from the same depth within each plot were combined and transported to the lab on ice. The homogenized soil samples were passed through a 2-mm sieve, and any visible root materials were separated and the soils were stored at 4°C until analysis. Above-ground litter input at knotweed site was estimated by harvesting the biomass from the invaded and noninvaded plots at the end of growing season before the tissue senescence. The annual above-ground litter input at kudzu site was calculated from the litter input in six randomly selected 0.5-m^2 plots at the end of the growing season. The root biomass at both the study site was estimated from the roots retrieved from the soil cores during the above sampling.

2.2 | Size fractionation of bulk soil

We adopted a soil size fractionation to differentiate between soil aggregate fractions and silt-clay fraction. Under the influence of organic matter and microbial biomass, the soil mineral particles are initially bound together to form microaggregates which then combine to form macroaggregates (Six, Paustian, Elliott, & Combrink, 2000). Based on the SOM composition, these aggregate fractions offer varying level of protection from decomposition to plant- and/or microbial-derived products through occlusion (Cotrufo et al., 2015). The soils were separated into different size fractions such as macroaggregates (250–2,000 μm), microaggregate (53–250 μm), and silt-clay (<53 μm) fractions as described in Elliott (1986; see Appendix S1). The C/N of the different soil fractions and the bulk soil were determined using an elemental analyzer (Carlo Erba NA 1500 Elemental Analyzer; Thermo Scientific, Lakewood, NJ, USA). The different size fractions from both soil depths (0–5 and 5–10 cm) were subjected to the analyses outlined below.

Because of the chemical complexity of SOM, we used a combination of wet chemistry and spectroscopy analysis that provided complementary data (Simpson & Simpson, 2012) to elucidate the composition of soil organic matter. The wet chemistry methods involved solvent extraction (free lipids), base hydrolysis (bound lipids and phenolics), and CuO oxidation (lignin monomers) followed by gas chromatography-mass spectrometry analysis to measure the individual plant and microbial biomarkers in soil. As wet chemistry techniques can characterize only <15% of the soil organic matter, we used spectroscopy methods such as the diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy to characterize the bulk organic

C-chemistry. The NMR spectroscopy was used as the ratios of functional groups obtained from the NMR spectroscopy further provides the degradation state of soil C (Baldock et al., 1997).

2.3 | Diffuse reflectance infrared Fourier transform spectroscopy analysis

The DRIFT spectra of the silt-clay and microaggregate fractions were collected using a Thermo Nicolet FTIR spectrometer with a DRIFT unit (Suseela et al., 2016; see Appendix S1). The background interfering signals from soil minerals were eliminated by subtracting the sample spectra from the spectra of the respective chemically oxidized (using 10% hydrogen peroxide) organic C-free soil fraction (Favilli et al., 2008). The particle size of the macroaggregate fraction was not evenly reduced below 40 μm after repeated ball milling resulting in a nonhomogenous representation of the bulk sample, and hence, this fraction was not used for the DRIFT analysis. Ten mg of the ball-milled sample was mixed with 140 mg of KBr and further ground in an agate motor and pestle into a fine powder (<40 μm). The sample dilution scheme was optimized based on preliminary experiments that were focused on spectral quality. The finer particle size of the sample and its dilution with KBr ensured minimal true reflectance and diffused-specular reflectance of the incident IR beam, and a deeper penetration of the IR beam within the sample matrix that facilitated true diffuse reflectance. The spectra were collected at a resolution of 4 cm^{-1}, and 64 scans were averaged to obtain a representative spectrum (Tharayil et al., 2011). The measured sample reflectance was converted into Kubelka Munk units to compensate for a stronger than expected absorption from a weak IR band, and to obtain a linear relationship between sample concentration and the corresponding spectral data. The sum of the peak areas at 2,930 and 2,870 cm^{-1} that represent aliphatic CH stretching (Haberhauer & Gerzabek, 1999; Leifeld, 2006; Niemeyer, Chen, & Bollag, 1992) was used as an indicator of the abundance of alkyl C in soil samples (Suseela, Tharayil, Xing, & Dukes, 2014; Suseela et al., 2013).

2.4 | Biomarker analysis

2.4.1 | Solvent extraction of free lipids

Each soil size fraction (300 mg) was combined with 3 ml of methanol (100%) and placed in an ultrasonic bath for 30 min. At the end of the sonication, the tubes were centrifuged, and the supernatant was transferred to a glass tube. The soil residue was sequentially extracted with 3 ml of dichloromethane/methanol (1:1 v/v) followed by 3 ml of dichloromethane (100%) using the above procedure (Otto & Simpson, 2007; Tamura & Tharayil, 2014). The above three sequential extracts from the same soil fraction were combined, and 7 ml of deionized water was added to induce phase separation and gently mixed by shaking. The bottom layer of dichloromethane was collected and dried completely under nitrogen and stored at −20°C until analysis.

2.4.2 | Base hydrolysis of bound lipids and phenolics

The residual soil from the above solvent extraction was combined with 3 ml of 1 N methanolic sodium hydroxide (NaOH) and incubated at 95°C for 3 hr. After cooling, the tubes were centrifuged, and the supernatant was transferred to a glass tube. The soil residue in the tube was then sequentially extracted first with 2 ml of 100% methanol and then with 2 ml of methylene chloride by sonicating for 30 min, and the supernatant from each extract was combined (Otto & Simpson 2007; Tamura & Tharayil, 2014). To the pooled extract (from three sequential extractions), 100 μl of nonadecane in 100% methanol (100 μg/ml) was added as an internal standard. The sample was acidified using 2 ml of hydrochloric acid (50%). The methylene chloride was phase-separated by adding 7 ml of deionized water and was collected and stored at −20°C until analysis.

2.4.3 | CuO oxidation of lignin monomers

Lignin is predominantly composed of three monophenolic units—vanillyl (V), syringyl (S), and cinnamyl (C) monomers. To measure the content of these lignin monomers, we followed the CuO oxidation method. The residual soil remaining after base hydrolysis was combined with 1.0 g copper oxide (CuO) and 150 mg ferrous ammonium sulfate in a 23-ml Teflon-lined acid digestion vessel. Fifteen ml of 2 N NaOH that had been previously sparged with argon for 20 min was added to the vessel and was sparged for an additional 15 min before closing each digestion vessel. The vessels were incubated at 160°C for 160 min. After cooling to room temperature, the content of the vessels was transferred to centrifuge tubes, and 100 μl of transcinnamic acid and ethylvanillin (200 μg/ml in 100% methanol) were added as internal standards. The samples were acidified using 3 ml of 18 N H_2SO_4 and centrifuged. The supernatant (12 ml) was transferred to glass tubes, and 2 ml of ethyl acetate was added. After cooling at 4°C for 15 min, the content was kept in a rotary mixer for gentle end-to-end mixing. The ethyl acetate fraction was collected after centrifugation and stored at −20°C until further analysis (Tamura & Tharayil, 2014; Wang, Tharayil, Chow, Suseela, & Zeng, 2015). Together with the total VSC lignin content, we also calculated the acid/aldehyde (Ad/Al) ratios of syringyl ([Ad/Al]s) and vanillyl ([Ad/Al]v) monomers of lignin which are measures of the degradation stage of lignin where higher ratios indicates progressing degradation (Otto & Simpson, 2007).

The extracts from solvent extraction (free lipids), base hydrolysis (bound lipids and phenolics), and CuO oxidation (lignin monomers) were reconstituted separately in 1 ml of methanol/methylene chloride (1:1) solution and analyzed using gas chromatography-mass spectrometry (GC-MS) for biomarker analysis (see Appendix S1). The content of the biomarkers was expressed as per gram organic C in soil size fractions for comparison between samples.

2.5 | Solid-state ^{13}C NMR spectroscopy analysis

For the analysis of soil samples using NMR, it was necessary to remove the paramagnetic material in the samples using 10% hydrofluoric acid (Schmidt, Knicker, Hatcher, & Kogel-Knabner, 1997; Appendix S1). Solid-state CrossPolarization Magic Angle Spinning (CP-MAS) NMR spectra were acquired using the parameters as per Clemente, Simpson, and Simpson (2011). The spectra were analyzed within five chemical shift regions (Mao et al., 2000; Preston, Trofymow, & The Canadian Intersite Decomposition Experiment Working Group, 2000; Clemente et al., 2011): alkyl (0–50 ppm; waxes, cutin, suberin, and lipids), O-alkyl (50–110 ppm; methoxy and ethoxy C in lignin and methoxy C in carbohydrates), aromatic and phenolic (110–165 ppm; aromatic C found in lignin, with possible contributions from protein and black carbon), carbonyl and carboxyl (165–220 ppm). The degradation state of C in soils was evaluated by calculating the ratio of alkyl C/O-alkyl C, which increases with progressive decomposition (Baldock et al., 1997; Sjogersten, Turner, Mahieu, Condron, & Wookey, 2003).

2.6 | Statistical analysis

Due to the differences in biotic and abiotic factors between the knotweed and kudzu sites, no statistical comparisions were made across the two sites, and comparisions were made between the invaded and adjacent noninvaded stands within each site that received an input of litter with contrasting chemistry. To compare the main and interactive effects of zones (invaded [IN] and adjacent noninvaded [OUT]) and soil size fractions (silt-clay, microaggregate, and macroaggregate) on the content of total organic C, alkyl C, VSC lignin, (Ad/Al)s ratio, (Ad/Al)v ratio, and plant and microbial biomarkers, we used a mixed-model restricted maximum likelihood analysis. Within a site, the invaded stands ($n = 6$) which represented independent experimental units were included as a random factor for these analyses. Statistically significant differences ($\alpha < .05$) were further subjected to Tukey's HSD multi-comparison test. All analyses were conducted using SAS (SAS version 9.2; SAS Institute, Cary, NC, USA).

3 | RESULTS

3.1 | Soil C quantity

At the knotweed site, soils under *P. cuspidatum* received ~2.5 times more litter input, which had one-third of the nitrogen per unit of C compared with that of the native litter from adjacent noninvaded soils inhabited by grasses and forbs (Table S1). At the kudzu site, as *P. lobata* was found to be invading established pine stands, both invaded and adjacent noninvaded soils received similar inputs of pine litter, and the invaded soils received an additional input of *P. lobata* litter, which had 6.5 times more nitrogen per unit of C than that in the pine litter.

At both sites, the effect of invasion on soil C content across different soil size fractions was evident at a 0–5 cm depth (Figure 2). At the knotweed site, the macroaggregate fraction at a 0–5 cm depth under *P. cuspidatum* had a 30% higher C content than that in the adjacent noninvaded soils (Figure 2a). However, the quantities of C within the silt-clay and microaggregate fractions were similar in the invaded and noninvaded soils. The macroaggregates had a ~30% higher C content than did the microaggregate and silt-clay fractions in the invaded soils, whereas in the noninvaded soils, the content of C in the microaggregate fraction was ~20% lower than that in the silt-clay and macroaggregate fractions (Figure 2a; $p < .01$). At a 5–10 cm depth, regardless of invasion zones, the microaggregate fractions had a ~40% lower C content compared with that in both silt-clay and macroaggregate fractions (Figure 2b; $p < .001$), a pattern that resembled the C abundance pattern in the noninvaded zones at a 0–5 cm depth.

At the kudzu site, the soils under *P. lobata* had 20% lower C content across all three size fractions at a 0–5 cm depth than did soils under noninvaded pine stands (Figure 2c; $p = .001$). This decrease in C content was more evident in the silt-clay and macroaggregate fractions. The soils at a 5–10 cm depth under noninvaded pine stands had higher quantities of C in the silt-clay and microaggregate fractions than in the macroaggregate fraction (Figure 2d; $p = .04$), whereas the C content of soils under *P. lobata*-invaded stands was similar across all size fractions.

3.2 | Abundance of alkyl C (DRIFT spectroscopy analysis)

At the knotweed site, the abundance of alkyl C at a 0–5 cm depth of the invaded and noninvaded soils varied as a function of soil size fraction (Figure 3a; $p = .004$). In the soils invaded by *P. cuspidatum*, the microaggregate fraction had two times higher alkyl C than that in the silt-clay fraction. Moreover, the alkyl C in the microaggregate fraction in the invaded soils was twice that in the same fraction in the adjacent noninvaded soil (Figure 3a). Within the noninvaded zone, both silt-clay and microaggregate fractions had similar amounts of alkyl C. At a 5–10 cm depth, irrespective of the invasion zones, the fraction of alkyl C in the silt-clay was 40% higher than that of the microaggregate fraction ($p = .02$; Figure 3b).

At the kudzu site, in the top 5 cm depth, the silt-clay fraction contained 41% higher alkyl C than the microaggregates, regardless of zone (Figure 3c; $p = .005$). Even though both invaded and noninvaded soils received a similar amount of pine litter, the soil size fractions under *P. lobata* had only one-third the amount of alkyl C in the noninvaded pine soil (Figure 3c; $p < .001$). At a 5–10 cm depth, the soils under *P. lobata* had less alkyl C in the microaggregate fraction than in the silt-clay fraction ($p < .05$). Similar to the results for the top soils, microaggregates at a 5–10 cm depth under *P. lobata* had lower amount of alkyl C than that in the noninvaded pine soils (Figure 3d; $p = .01$).

3.3 | Abundance of lignin monomers

At the knotweed site, the concentration of VSC lignin phenols (the sum of V-vanillyl, S-syringyl, and C-cinnamyl phenols) across soil fractions at a 0–5 cm depth varied among invasion zones ($p = .04$;

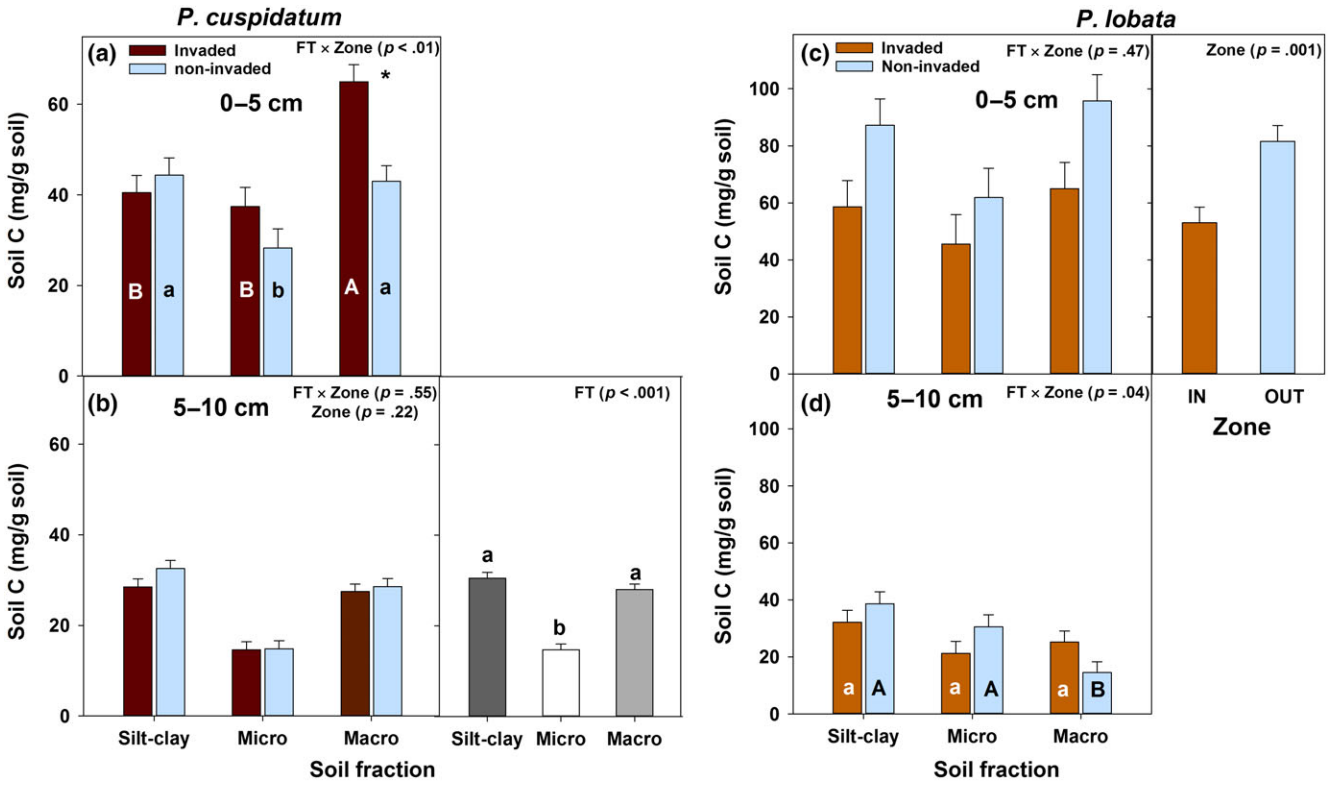

FIGURE 2 The amount of carbon in size fractions per gram of soil in the top 0–5-cm (a) and 5–10-cm soils (b) at knotweed (*Polygonum cuspidatum*) and (c) top 0–5-cm and (d) 5–10-cm soils at kudzu (*Pueraria lobata*) sites. Each bar represents mean ± *SE* ($n = 6$). Any significant main effect of treatments [soil size fractions, invasion zones] is shown in graphs on the right of the interaction graph. Asterisk represents differences in means (Tukey's honestly significant difference) within a soil size fraction. Letters "A" and "B" represent differences within invaded stands and letters "a" and "b" represent differences within the noninvaded stands. FT, fraction; micro, microaggregate; macro, macroaggregate; IN, invaded stands; out, adjacent noninvaded stands [Colour figure can be viewed at wileyonlinelibrary.com]

Figure 4a). Both silt-clay and microaggregate fractions under *P. cuspidatum* had ~40% lower concentrations of lignin compared with that in the noninvaded soils (Figure 4a), and the microaggregate fraction had a higher content of lignin across the zones. Similarly, at a 5–10 cm depth, relative to the noninvaded soils, soils under *P. cuspidatum* had lower lignin phenols in both the silt-clay and the microaggregate fractions (Figure 4b). Irrespective of the invaded and noninvaded zones, the microaggregates had a nearly 50% higher concentration of lignin phenols than that in the silt-clay fraction ($p < .01$; Figure 4b). At the knotweed site, the (Ad/Al)s and (Ad/Al)v ratios did not differ among the various factors at a 0–5 cm depth (Table S1). However, at a 5–10 cm depth, compared with the knotweed invaded stands, the noninvaded stands had higher ratios across the fractions. Similarly, the silt-clay fraction across both zones had higher (Ad/Al)s and (Ad/Al)v ratios than did the microaggregate fraction at a 5–10 cm depth, thus indicating greater degradation of lignin associated with the silt-clay fraction.

At the kudzu site, the concentration of lignin phenols across soil fractions varied with invasion zones at the top 0–5 cm depth ($p = .009$; Figure 4c). The concentrations of lignin monomers in the micro- and macroaggregate fractions under *P. lobata* were lower than those in the respective fractions in the noninvaded soils under pine stands. At a 5–10 cm depth, the concentration of lignin was higher in the microaggregate fraction compared with that in the silt-clay fraction in both invaded and noninvaded zones (Figure 4d). In the silt-clay fraction, the lignin monomer concentration in invaded soils under *P. lobata* was lower than that in the noninvaded *Pinus* soils ($p < .05$). At the kudzu site, the (Ad/Al)s and (Ad/Al)v ratios did not differ among the factors (Table S3).

3.4 | Extractable plant-derived biomarkers

At the knotweed site, at a 0–5 cm depth, the invaded soils under *P. cuspidatum* had a marginally higher content of leaf-derived cutin biomarkers in the macroaggregate and microaggregate fractions than in the adjacent noninvaded soils, whereas the cutin content of the silt-clay fraction was similar between the two zones (Figure 5a; $p = .06$). The cutin content was higher in soils under *P. cuspidatum* (Figure 5a; $p = .002$) across the size fractions. In contrast, the root-derived suberin had a marginally higher abundance in the noninvaded zone across the size fractions than that in soils under *P. cuspidatum* (Figure 5b; $p = .08$). Long-chain fatty acids (LFA), primarily derived from plant waxes, followed a trend similar to that of cutin biomarkers, in which macroaggregate and microaggregate fractions had higher LFA content than that in the silt-clay fraction across the zones (Figure 5c; $p < .01$). The LFA content was higher in

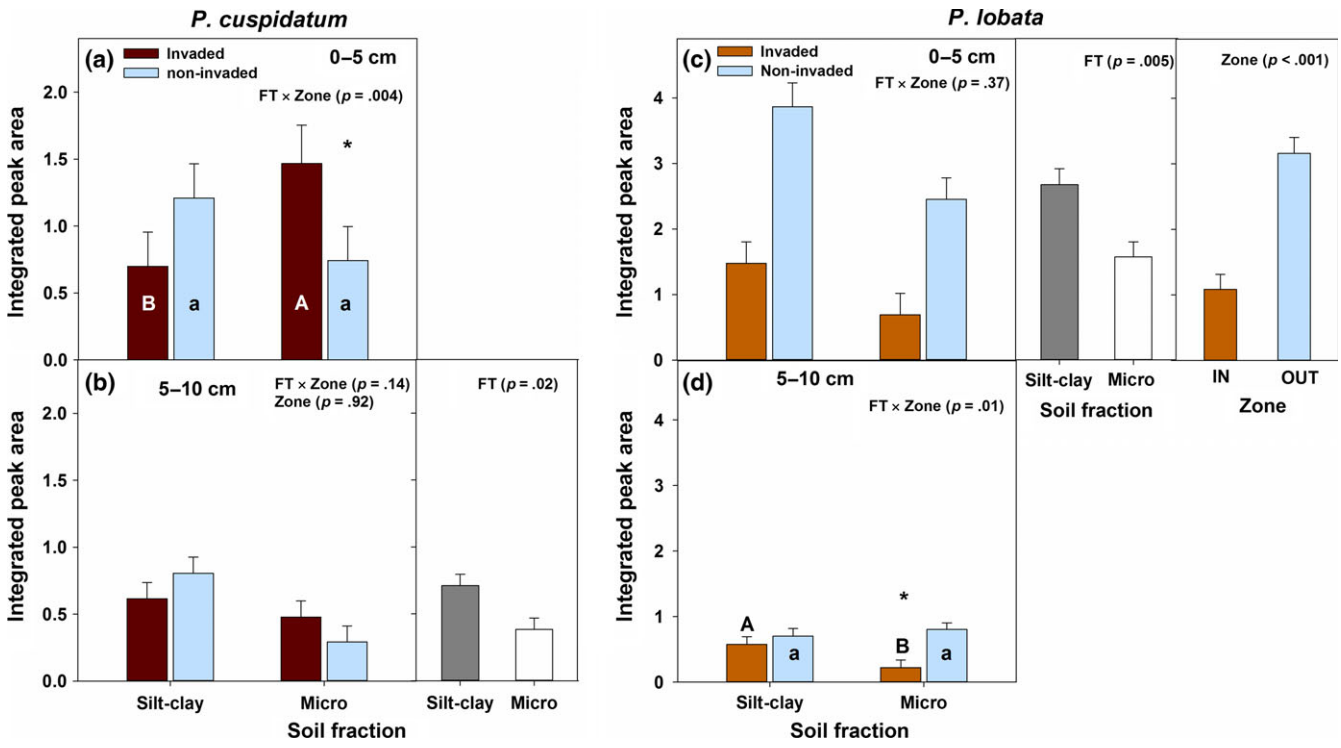

FIGURE 3 Area of the peaks (sum of 2,930 and 2,870 cm^{-1}) from diffuse reflectance infrared Fourier transform spectroscopy analysis corresponding to alkyl C (C–H symmetric and asymmetric stretches) in silt-clay and microaggregate fractions in the top 0–5-cm (a) and 5–10-cm soil (b) in knotweed (*Polygonum cuspidatum*) site and (c) top 0–5-cm and (d) 5–10-cm soils in kudzu (*Pueraria lobata*) site. Each bar represents mean ± *SE* ($n = 6$). Any significant main effect of treatments [soil size fractions, invasion zones] is shown in graphs on the right of the interaction graph. Asterisk represents differences in means (Tukey's honestly significant difference) within a soil size fraction. Letters "A" and "B" represent differences within invaded stands and letters "a" and "b" represent differences within the noninvaded stands. FT, fraction; micro, microaggregate; IN, invaded stands; Out, adjacent noninvaded stands [Colour figure can be viewed at wileyonlinelibrary.com]

P. cuspidatum-invaded stands than in the noninvaded soils across the different soil size fractions (Figure 5c; $p = .003$). The concentration of phytosterol in the different soil size fractions varied among zones (Figure 5d; $p = .009$) and followed a pattern similar to those of the cutin and LFA biomarkers. The concentrations of phytosterol in the macroaggregate and microaggregate fractions were higher in soils under *P. cuspidatum* than in the respective fractions in the noninvaded soil (Figure 5d; $p = .009$). Within the *P. cuspidatum*-invaded soil, the concentrations of phytosterol were three times higher in the macroaggregate and microaggregate fractions than in the silt-clay fraction ($p = .03$). At a 5–10 cm depth, the abundance of cutin, suberin, and LFA did not vary with soil size fractions or zones ($p > .05$) except for phytosterol (Fig. S2), which had a higher concentration in the silt-clay fraction than in the microaggregate fraction regardless of invasion zone ($p = .04$).

At the kudzu site, at a 0–5 cm depth, the concentration of cutin in the silt-clay fraction was higher than that in the macroaggregate and microaggregate fractions across the zones (Figure 5e; $p = .002$). Across the size fractions, soils under *P. lobata* had a lower abundance of cutin biomarkers than did soils under noninvaded pine stands (Figure 5e; $p = .01$). Similarly, the soils under *P. lobata* had a lower concentration of all other quantified plant polymers, including suberin, LFA, and phytosterol, across all fractions, as compared with the concentrations in the adjacent noninvaded soils under pine stands (Figure 5f–h; $p < .05$). Generally, the abundance of these plant polymers was similar across the different size fractions in soils under noninvaded pine stands. At the kudzu site, at a 5–10 cm depth, the concentrations of cutin, suberin, and LFA were similar to the concentrations in the top 5 cm and were higher in the silt-clay fraction than in the microaggregate fraction, regardless of invasion zone ($p < .01$; Fig. S3).

3.5 | Microbial-derived biomarkers

At the knotweed site, the concentration of short-chain fatty acids (constituents of plant/microbial biomass and products; Otto, Shunthirasingham, & Simpson, 2005) varied with size fraction across invasion zones; the microaggregate fraction had a higher content of SFA than did the silt-clay fraction (Figure 6a; $p = .02$). The concentration of short-chain fatty acids at a 5–10 cm depth was not influenced by size fraction or invasion zone ($p > .05$). At the knotweed site, in both invaded and noninvaded soils, the silt-clay fraction had the lowest ergosterol content, and the content of ergosterol increased threefold from micro- to macroaggregates ($p = .02$; Figure 6b). In both aggregate fractions, the soils under *P. cuspidatum* had lower ergosterol concentrations than did the noninvaded zone

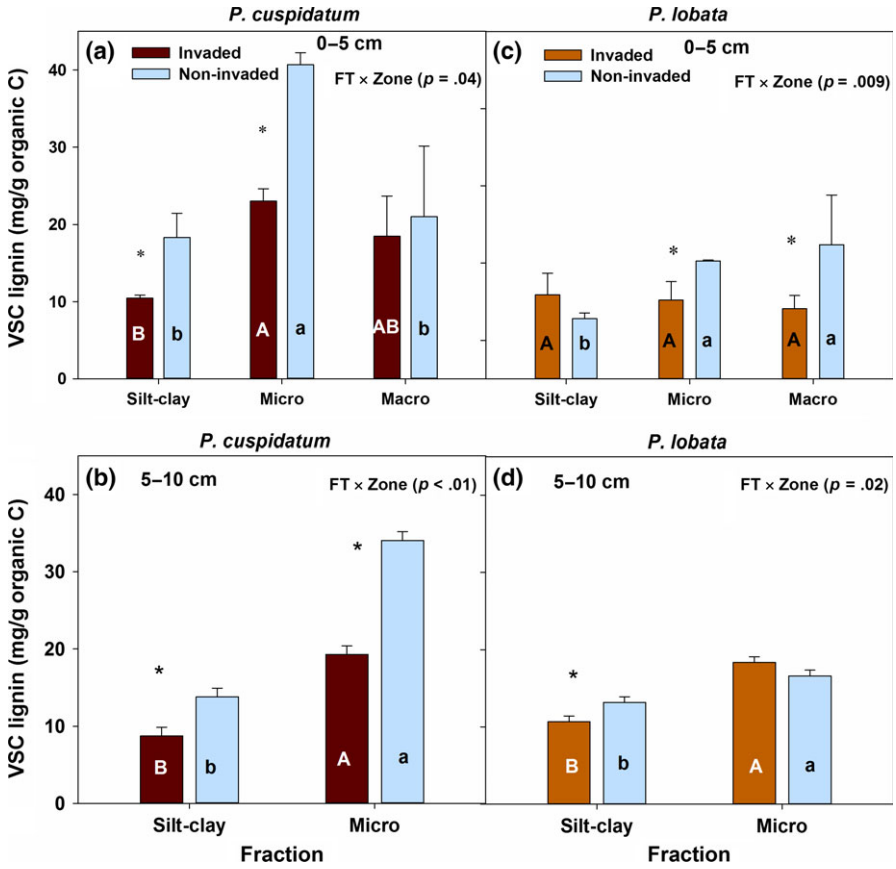

FIGURE 4 Content of VSC lignin monomers (per gram organic carbon in soil size fractions) after CuO oxidation at *Polygonum cuspidatum* (a and b) and at *Pueraria lobata* (c and d) sites in the top 0–5-cm and 5–10-cm, respectively. "VSC" represents the sum of syringyl, vanillyl, and cinnamyl monomers of lignin. Each bar represents mean ± *SE* (n = 6). Asterisk represents differences in means (Tukey's honestly significant difference) within a soil size fraction. Letters "A" and "B" represent differences within invaded stands and letters "a" and "b" represent differences within the noninvaded stands. FT, fraction; micro, microaggregate; macro, macroaggregate [Colour figure can be viewed at wileyonlinelibrary.com]

(Figure 6b; p < .01). However, the ergosterol content was higher in the invaded zone than in the noninvaded zone at a 5–10 cm depth across the soil size fractions (Figure 6c; p = .049).

At the kudzu site, the concentration of short-chain fatty acids was similar across the soil size fractions in the noninvaded zone, whereas silt-clay and macroaggregate fractions had higher SFAs than those in microaggregates in soils under *P. lobata* (p = .04; Figure 7a). Within the macroaggregate fraction, soils under *P. lobata* had a higher abundance of SFA than did the respective fraction under non-invaded pine stands (Figure 7a). At 5–10 cm depth, across the invasion zones, the silt-clay fraction contained more than twice the amount of short-chain fatty acids than that in the microaggregate fraction (p < .01; Figure 7b). The concentration of ergosterol under *P. lobata* was less than half the value observed in the noninvaded soils in aggregate fractions at the top 5 cm depth (p = .003; Figure 7c). The invaded soils under *P. lobata* had less ergosterol than did the noninvaded pine stands at a 5–10 cm depth regardless of size fraction (p < .01; Figure 7d). Similarly, the content of ergosterol was higher in the silt-clay fraction than in the microaggregate fraction across the invasion zones.

3.6 | NMR analyses

At the knotweed site, the silt-clay fraction had lower aromatic and phenolic C than did the microaggregate fraction across the zones (Table 1). In the microaggregate fraction, the ratio of alkyl C to O-alkyl C was higher in *P. cuspidatum*-invaded soils than in the noninvaded soils. At the kudzu site, the silt-clay fraction across both zones had a higher content of alkyl C and a higher ratio of alkyl C to O-alkyl C than the microaggregate fraction, thus indicating an advanced stage of SOM degradation in the silt-clay fraction.

4 | DISCUSSION

4.1 | Influence of litter chemistry on the quantity of mineral-associated soil C

During decomposition of plant litter, microbial synthesis of less complex biomolecules accompanies the initial mineralization of the tissue matrix (Lehmann & Kleber, 2015; Prescott, 2010; Schmidt et al.,

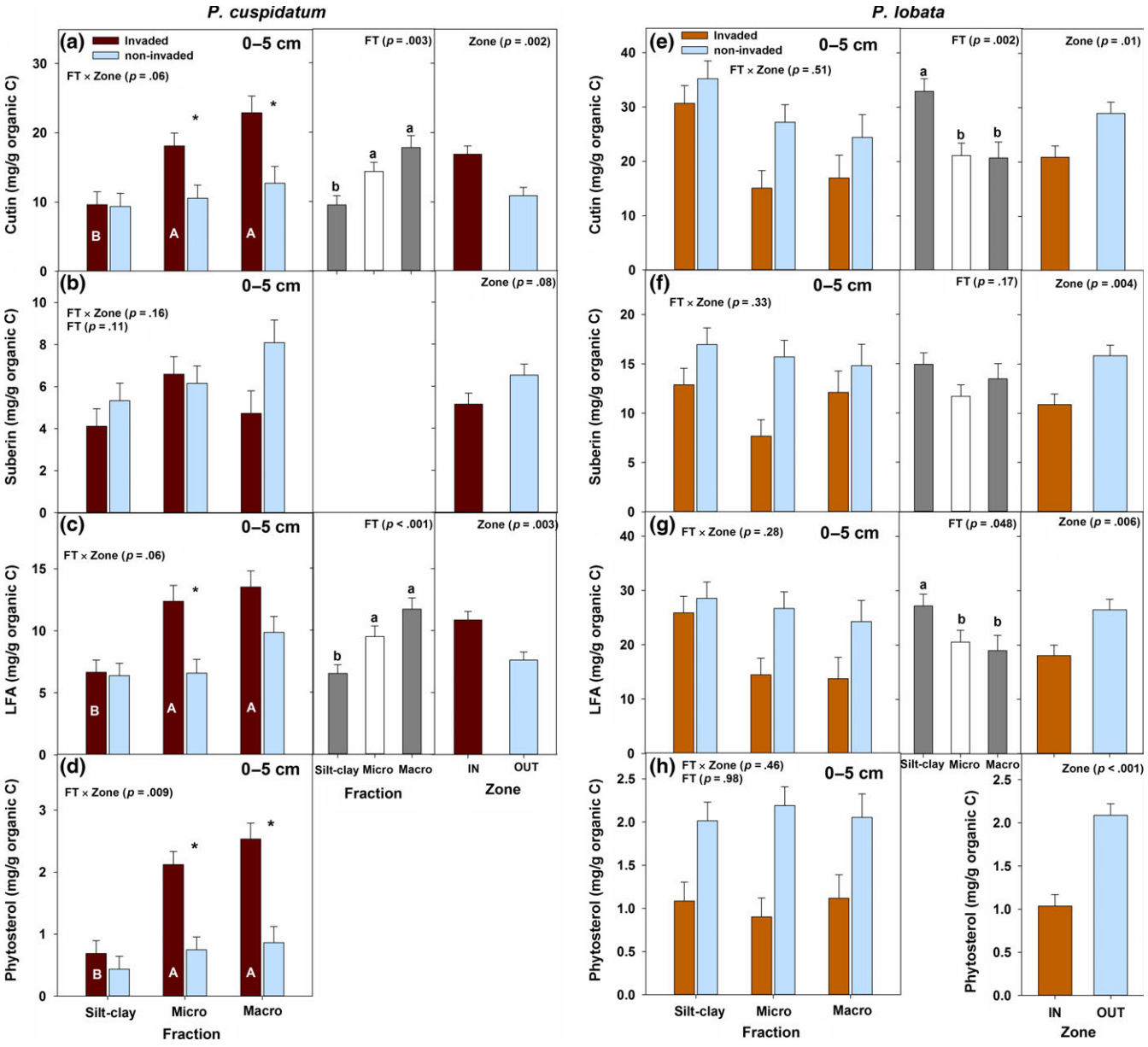

FIGURE 5 The content of plant biomarkers (per gram organic carbon in soil size fractions; (a) ∑Cutin, (C_{14}–C_{18} hydroxyalkanoic acid, C_{16}-di-hydroalkanoic acid, ω-hydroxy- and ω-hydroxy-epoxy alkanoic acids (C_{16}–C_{18})); (b) ∑Suberin, (α,ω-dicarboxilic acids (C_{16}–C_{24}; saturated and substituted) and ω-hydroxyalkanoic acids (C_{20}–C_{30}; saturated and substituted)); (c) ∑LFA, long-chain fatty acids (>C_{24} alkanes, >C_{22} *n*-alkanoic acids and alkanols); (d) ∑Phytosterols extracted from the silt-clay, micro- and macroaggregate fractions in the top 0–5-cm soil of *Polygonum cuspidatum* and (e) ∑Cutin, (f) ∑Suberin, (g) ∑LFA and (h) ∑Phytosterols extracted from the silt-clay, micro-, and macroaggregate fractions in the top 0–5-cm soil of *Pueraria lobata sites*. Each bar represents mean ± *SE* ($n = 6$). Any significant main effect of treatments [soil size fractions, invasion zones] is shown in graphs on the right of the interaction graph. Asterisk represents differences in means (Tukey's honestly significant difference) within a soil size fraction. Letters "A" and "B" represent differences within invaded stands and letters "a" and "b" represent differences within size fractions across zones. FT, fraction; micro, microaggregate; macro, macroaggregate; IN, invaded stands; Out, adjacent noninvaded stands [Colour figure can be viewed at wileyonlinelibrary.com]

2011). The greater reactive surfaces of these microbial products (Golchin, Baldock, & Oades, 1998) potentially facilitate their self-assembly (chemisorption) into narrow mineral lattices (Kleber et al., 2007). However, the larger molecular size and the associated steric hindrance may prevent the association of plant macromolecules with the inner-surfaces of minerals. Therefore, we hypothesized that the input of litter rich in recalcitrant compounds that slows the production of microbial products would subsequently lead to the accrual of more C in soil aggregate fractions than in the silt-clay fraction. This hypothesis was supported by the greater C content of the macroaggregate fraction in soils under *P. cuspidatum* that received recalcitrant litter (Figure 2a). The lower C content of the silt-clay fraction in soils under *P. cuspidatum* (Figure 2a), despite the higher input of litter to this soil, may potentially have resulted from

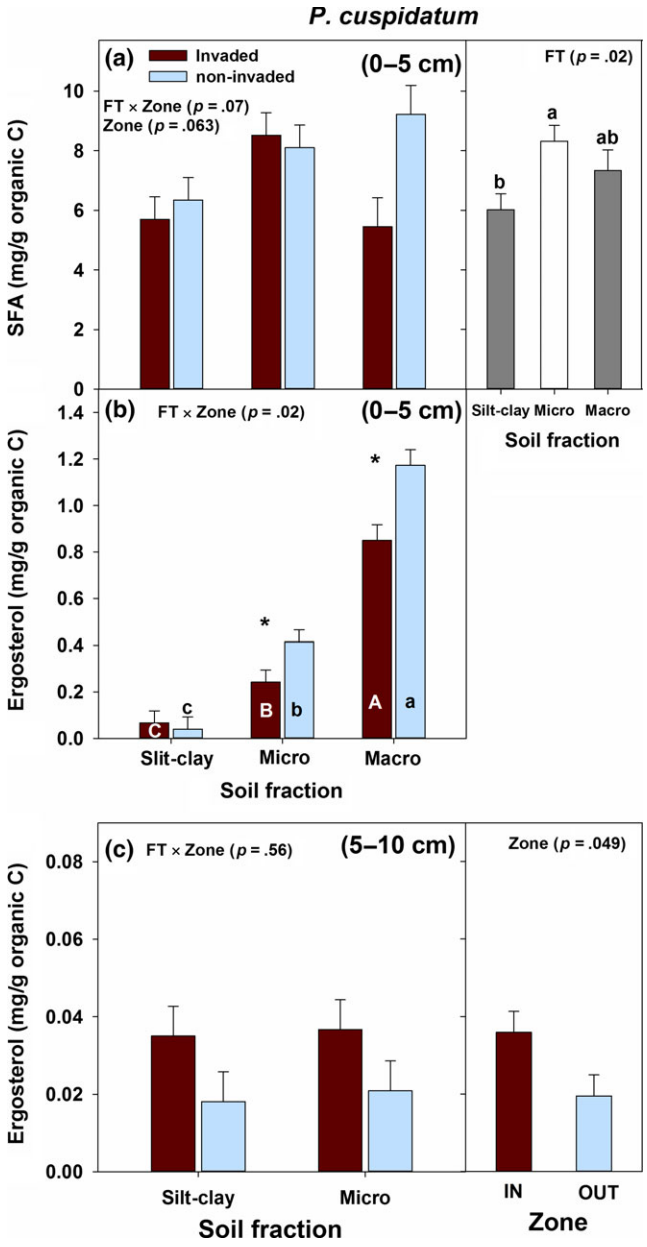

FIGURE 6 Extractable microbial biomarkers (per gram organic carbon in soil size fractions) in *Polygonum cuspidatum*-invaded and adjacent noninvaded soils. (a) short-chain fatty acid (ΣSFA; C10–C18 *n*-alkanoic and *n*-alkanoic acid) in 0–5 cm depth, and (b and c) ergosterol in 0–5 and 5–10 cm depth, respectively. Each bar represents mean ± *SE* (*n* = 6). Any significant main effect of treatments [soil size fractions, invasion zones] is shown in graphs on the right of the interaction graph. Asterisk represents differences in means (Tukey's honestly significant difference) within a soil size fraction. In (b) Letters "A" and "B" represent differences within invaded stands and letters "a" and "b" represent differences within noninvaded stands. FT, fraction; micro, microaggregate; macro, macroaggregate; IN, invaded stands; Out, adjacent noninvaded stands [Colour figure can be viewed at wileyonlinelibrary.com]

both the lower rate of mineralization of *P. cuspidatum* litter (Suseela et al., 2013) and the lower ability of plant compounds to associate with the soil mineral fraction (Grandy & Neff, 2008). The sequestration of C in the macroaggregate fraction of *P. cuspidatum*-invaded soils may have been partly facilitated by the abundance of fungi, as evident from the two times higher ergosterol content in the macroaggregate fraction than in the microaggregate fraction (Figure 6b). Microbial products, including fungal hyphae and biofilms (Daynes, Field, Saleeba, Cole, & Mcgee, 2013), promote the formation of aggregates in soil (Courtier-Murias et al., 2013; Golchin et al., 1998; O'Brien & Jastrow, 2013), thus protecting the adsorbed SOM by occlusion (Dungait, Hopkins, Gregory, & Whitmore, 2012; Martens, Reedy, & Lewis, 2004). Additionally, fungal mycelium may also serve as a source of SOM (Clemmensen et al., 2013). Previous studies have reported similar trends, wherein the invasion of woody shrubs that input recalcitrant litter into native grassland results in the accrual of soil C, primarily in the aggregate soil fractions (Filley, Boutton, Liao, Jastrow, & Gamblin, 2008; Lorenz, Lal, Preston, & Nierop, 2007). Similar results have also been reported from multiple field-level detrital manipulation experiments in which the aggregate associated carbon, and not the mineral-associated carbon, has been found to be influenced by litter addition treatments even after 50 years of continuous litter manipulation (Lajtha et al., 2014). Our results indicated that the previously reported higher bulk soil C content in *P. cuspidatum*-invaded soils (Tamura & Tharayil, 2014) was attributable to an abundance of particulate soil organic matter mainly contributed by the higher input of aboveground litter by this species. Inputs of *P. cuspidatum* litter did not influence the content of C in soil size fractions at a 5–10 cm depth, potentially because of the lower amount of dissolved organic C percolating below the top soil, owing to the lower rate of decomposition of this litter (Suseela et al., 2013). A similar lesser influence of *P. cuspidatum* on soil N cycling at a 5–10 cm depth has previously been observed across several *P. cuspidatum*-invaded sites (Tharayil et al., 2013).

Because the input of labile litter facilitates faster decomposition and accumulation of microbial products, at the knotweed site, we expected to observe a greater content of C in the silt-clay fraction in soils under the native species that input labile-rich litter. However, this hypothesis was not supported, and the quantity of C in the silt-clay fraction at the knotweed site was similar across the invasion zones. Moreover, the C content of the silt-clay fraction in the noninvaded zone was similar to that in the macroaggregate fraction (Figure 2a). These deviations may potentially have been because of the soil mineral properties of the silt-clay surfaces (Hassink, 1997) at the knotweed site. Even when the microbial products that can form strong organo-mineral associations are nonlimiting, the formation of stable soil organic C is limited by the capacity of soils to sequester C in the mineral matrices (C saturation deficit; Castellano, Mueller, Olk, Sawyer, & Six, 2015). At the knotweed site, the soil was characterized by a low clay content (5.8%) with a low surface area (29.54 m^2/g), a condition that may potentially limit the association of SOM to mineral surfaces beyond a threshold.

At the kudzu site, the input of *P. lobata* litter into pine stands could accelerate the degradation of pine-derived resident SOM by facilitating microbial co-metabolism. However, because of a strong linkage of C chemisorbed to the silt-clay fraction, we hypothesized

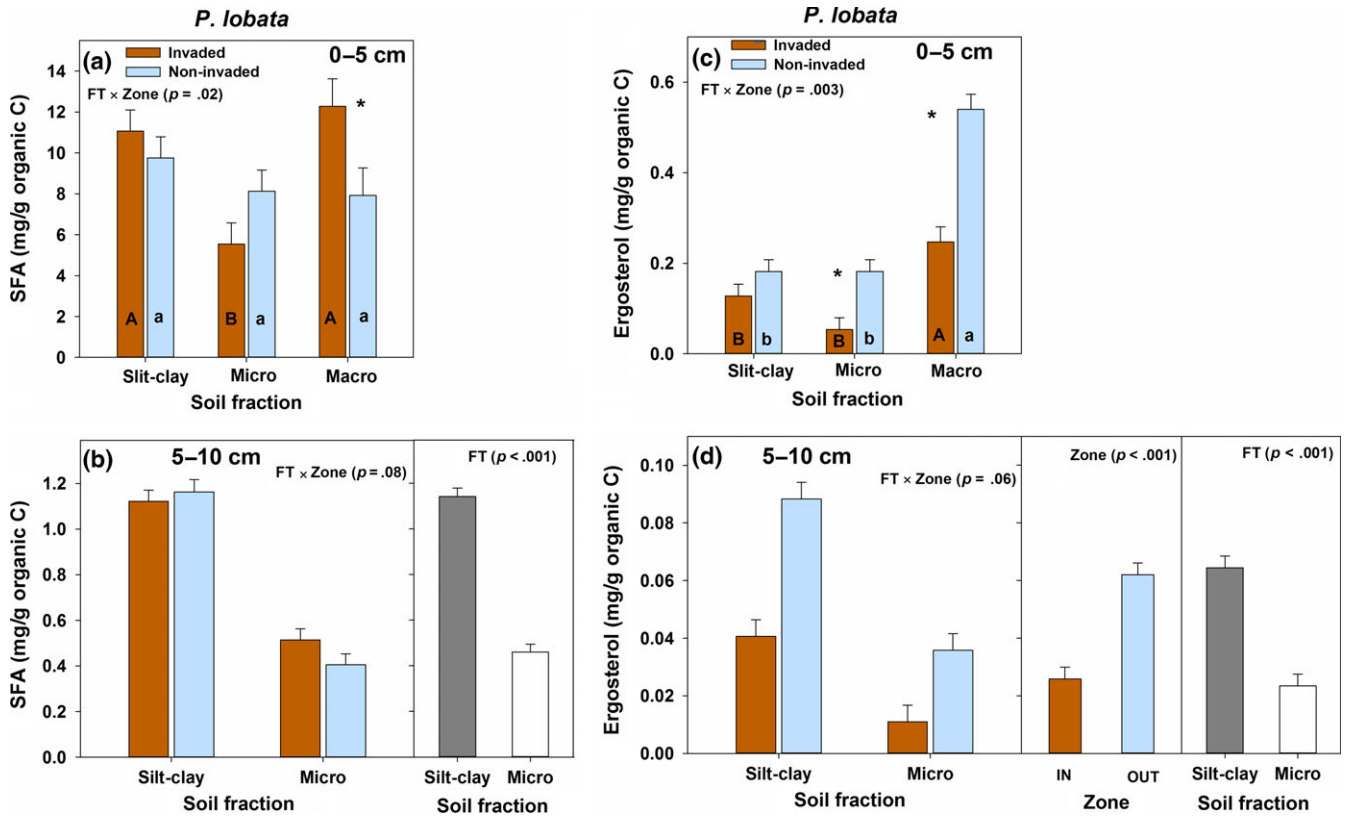

FIGURE 7 Extractable microbial biomarkers (per gram organic carbon in soil size fractions) in *Pueraria lobata*-invaded and adjacent noninvaded soils. (a and b) Short-chain fatty acid (ΣSFA; C10–C18 *n*-alkanoic and *n*-alkanoic acid) and (c and d) ergosterol in soil size fractions at 0–5 and 5–10 cm, respectively. Each bar represents mean ± *SE* ($n = 6$). Any significant main effect of treatments [soil size fractions, invasion zones] is shown in graphs on the right of the interaction graph. Asterisk represents differences in means (Tukey's honestly significant difference) within a soil size fraction. Letters "A" and "B" represent differences within invaded stands and letters "a" and "b" represent differences within noninvaded stands. FT, fraction; micro, microaggregate; macro, macroaggregate; IN, invaded stands; Out, adjacent noninvaded stands [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Integration results from solid-state ^{13}C NMR spectroscopy with the relative percent of chemical shift regions corresponding to different carbon functional groups in soils at 0–5 cm depth from the invaded and noninvaded stands at the knotweed and kudzu site

	Relative percentage of ^{13}C NMR signal				
Soil sample	Alkyl C (0–50 ppm)	O-alkyl C (50–110 ppm)	Aromatic and phenolic C (110–165 ppm)	Carboxyl and carbonyl C (165–220 ppm)	Alkyl C/O-alkyl C
Polygonum cuspidatum					
Silt-clay					
Invaded	35	40	9	16	0.88
Noninvaded	36	41	9	14	0.88
Microaggregate					
Invaded	33	42	13	12	0.79
Noninvaded	30	42	15	13	0.71
Pueraria lobata					
Silt-clay					
Invaded	44	33	9	14	1.33
Noninvaded	43	32	12	13	1.34
Microaggregate					
Invaded	34	31	23	12	1.10
Noninvaded	36	34	16	14	1.06

that this C loss due to positive-priming effect would be lower for C stored in silt-clay fractions compared to the aggregate fractions. Contrary to our prediction, the soils under *P. lobata* that received similar amounts of pine litter as those in the noninvaded stands had lower C content across all size fractions. Although the mechanisms driving this observation were not determined by our study, we postulate that this low amount of C across the soil size fractions under *P. lobata* may be partially because of the ability of labile inputs to desorb C from the organo-mineral associations. For example, the input of oxalic acid into soils has been shown to increase the C loss from mineral associations by complexation and dissolution reactions (Keiluweit et al., 2015). Soils at the kudzu site had a high content of clay (14%) and iron oxides with a high surface area (179 m^2/g), both of which provide high C loading capacity via ligand exchange (Dixon & Weed, 1989; Kleber, Mikutta, Torn, & Jahn, 2005). However, under a high amount of C loading, organic compounds that are sorbed far from mineral surfaces are susceptible to microbial decomposition because of fewer ligand attachments with iron oxides (Kaiser & Guggenberger, 2000).

Despite the higher input of litter, the lower amount of C under *P. lobata* soil mirrors the accelerated decomposition of recalcitrant plant litter when present in mixtures of labile litter (Gartner & Cardon, 2004) as well as the degradation of mineral-associated soil C, which is facilitated by root exudates (Drake et al., 2011; Phillips, Finzi, & Bernhardt, 2011). Previous studies have reported that the invasion of grasses into hardwood forests has led to the loss of native C from the faster-cycling particulate organic matter because of microbial priming (Strickland, Devore, Maerz, & Bradford, 2010). Additionally, the fungal abundance under *P. lobata*-invaded stands decreased in the macroaggregate fraction despite an increase in microbial products, as indicated by SFA, which might also partly have contributed to the lower C at this site. Overall, our results highlighted the interaction between the chemistry of the plant litter and the association of C with soil size fractions and supported the framework of SOM formation proposed by Cotrufo et al. (2013, 2015).

4.2 | Influence of litter chemistry on the composition of mineral-associated soil C

The chemistries of the plant compounds and microbial residues, both of which differ in their propensity to associate with soil size fractions, influence the pathways of soil C stabilization. We hypothesized that the input of recalcitrant litter would increase the concentration of plant-derived C in the aggregate fractions more than in the silt-clay fraction. At the knotweed site, the *P. cuspidatum*-invaded soils that received recalcitrant litter were abundant in plant compounds in the aggregate fractions compared with the adjacent noninvaded soils, as evidenced by the higher overall abundance of alkyl C (Figure 3a). Even though this alkyl C may have been partly contributed by microbial fatty acids, the higher abundance of cutin, LFA, and phytosterols in the aggregate fractions of the invaded soils (Figure 5) supported the plant origin of alkyl C in aggregates. Therefore, the input of recalcitrant litter resulted in a larger proportion of plant-derived compounds in the physically protected soil aggregate fractions than in the silt-clay fraction under *P. cuspidatum*, thus supporting the SOM formation pathway proposed by Cotrufo et al. (2015). Unlike other components of plant litter, alkyl C compounds and plant sterols have demonstrated a relatively greater persistence in soil (Derenne & Largeau, 2001; Feng, Simpson, Wilson, Williams, & Simpson, 2008; Winkler et al., 2005); however, these compounds are still prone to microbial decomposition based on their association to lignocellulosic matrix (Angst, Heinrich, Kogel-Knabner, & Mueller, 2016). The slower rate of decomposition of recalcitrant plant compounds (Mueller et al., 2013), coupled with the overall higher input of these compounds in the *P. cuspidatum*-invaded stands, would explain the apparent persistence of these recalcitrant compounds in aggregate fractions. Moreover, the conditions that limit microbial activity in aggregate fractions, including insufficient oxygen and water (Von Lutzow et al., 2006), may further increase the residence time of this occluded C.

Lignin was abundant in the microaggregate fraction of the noninvaded soils at knotweed site (Figure 4a), a result contrary to our predictions and in contrast to the distribution of the plant polymers that were abundant in the aggregate fractions of soils under *P. cuspidatum*. The greater abundance of lignin in grass- and forb-dominated noninvaded soils may have partially resulted from the higher rooting density of these native species (Table S1). This observation was further supported by the observed trend of greater abundance of root-biopolymer suberin in the macroaggregate fraction of the noninvaded soils (Figure 5b). Lignins, which are also abundant in the roots, cross-link with proteins and cellulose and form a strong structural framework that decreases the rate of microbial decomposition (Carpita, 1996; Suseela et al., 2014). Additionally, the fibrous rooting architecture of the grasses would result in better soil aggregation, which in turn would offer greater protection to the occluded SOM. The twofold higher rooting density of the native grasses and forbs at a 5–10 cm depth may partly explain the similar increase in lignin content of noninvaded soils at this depth. The higher content of lignins may have contributed to the abundance of fungal biomass in the noninvaded soil at the knotweed site, as evidenced by the higher ergosterol observed in the aggregate fractions (Figure 6b). Therefore, although *P. cuspidatum* produced a higher amount of aboveground recalcitrant-rich litter, the deep-rooted native grasses and forbs produced significantly higher amounts of recalcitrant litter belowground, thus possibly resulting in the observed deviation from our initial prediction that associated contrasting vegetation type with C in the soil size fractions.

Of the various plant-derived compounds in the noninvaded soils under the *Pinus* stands, only lignin showed a soil size fraction-dependent abundance in which the lignin content was higher in the aggregate fractions of the noninvaded soils (Figure 4c). However, contrary to our hypothesis, other plant-derived compounds, such as cutin, suberin, and LFA, exhibited similar abundance across all soil size fractions in noninvaded soils (Figure 5e–g). This similar abundance of plant-derived compounds across the size fractions under the noninvaded *Pinus* stands contrasts with the observations in soils under

P. cuspidatum, where the silt-clay fraction had a lower abundance of plant biomarkers compared with that in the aggregate fractions. The main structural clay found in ultisols at the kudzu site is kaolinite, which is composed of a nonexpanding 1:1 phyllosilicate structure that has a relatively lower contribution to organic matter retention. However, clay fractions at the kudzu site were predominantly illite and iron and aluminum oxides and hydroxides, which provided a higher external surface area for sorption of SOM. This higher surface area contributed by sesquioxides may have resulted in a higher C loading of the silt-clay fraction in the kudzu site, regardless of the chemistry of plant input. This difference further highlights the importance of mineral properties in sequestering soil C, an influence as important as that of the litter chemistry (Castellano et al., 2015). Therefore, the effects of plant species on the accrual and composition of SOM are context dependent (De Deyn et al., 2008), and the influences of site characteristics, particularly soil mineralogy, may confound the direct effect of litter chemistry on the formation of SOM.

Despite a similar input of recalcitrant pine litter in both invaded and noninvaded soils, the invaded soils that received additional inputs of labile litter of *P. lobata* exhibited fewer plant-derived compounds such as alkyl C, cutin, suberin, long-chain fatty acids and phytosterols. These results suggested that the addition of labile litter resulted in the loss of recalcitrant plant compounds, regardless of the soil size fraction with which they were associated. This change in the composition of SOM under *P. lobata* was further supported by the lower abundance of fungal biomarkers in the invaded soils. The loss of phytosterols from the silt-clay fraction may have been attributed to their potential association to the external surfaces of sesquioxides, which were predominant at this site. Surface association of SOM on mineral surfaces, as opposed to their occlusion in inter- and intra-lattice spaces, predisposes them to losses because of the complexation and dissolution reactions (Keiluweit et al., 2015).

Litter chemistry, microbial physiology, soil mineral properties, and their interactions largely regulate the C sequestration potential in soils. Our study investigated the formation of SOM along a plant–microbial–mineral continuum (Figure 1) in two unmanaged ecosystems, each of which received two contrasting litter chemistries within the same climatic and edaphic envelope. Notably, as in most studies in invasion ecology, the invasion was not experimentally manipulated at our study sites, and hence, the differences in soil C that we found between invaded and noninvaded soils may have preceded rather than resulted from invasion. However, similar patterns of soil N (Tharayil et al., 2013) and C chemistry (Suseela et al., 2016) have been observed in invaded soils across regional scales, thus supporting the idea that many of these soil processes are driven by invasive species at our study sites. Generally, our study showed that the input of litter rich in recalcitrant compounds resulted in an accrual of SOM, which was abundant in plant polymers, in soil aggregate fractions. However, the association of the plant polymers with soil size fraction was also influenced by the soil mineralogy, where the mineral matrix with a greater surface area potentially permitted a greater association of plant polymers. Our results also showed that the rooting pattern and the composition of belowground litter may have had an overriding influence on the effect of the aboveground litter on soil C, such that greater rooting density and rooting depth overrode the input of labile aboveground litter and resulted in a greater accumulation of recalcitrant plant compounds in the aggregate fraction. Previous studies have shown that even when the input of labile C promotes the loss of the resident SOM through microbial priming, the higher plant productivity that follows the accelerated nutrient cycling would nullify or overcompensate for this loss of soil C (Kogel-Knabner, 2017). Thus, the reported C loss under labile input in our study may have been highly specific to the edaphic and climatic environment, such that C loss occurred under conditions that promoted faster decomposition of plant-derived SOM, but concomitantly restricted the mineral association of the decomposition products, along with a nonsignificant change in the overall productivity of the system. These results provide a mechanistic framework that could partly explain the context dependency of the influence of an invasive species on the soil C content, where the net magnitude and direction of an exotic species on soil C storage across its invaded ranges would be strongly regulated by multiple biotic and abiotic factors including the chemical and physical traits of native species that are being displaced, soil microbial community and climate that regulate the rate and type of decomposition products, and edaphic factors including soil mineralogy. Overall, our study provides insight into the importance of considering the complex interactions among plant litter chemistry, physiology of the heterotrophic soil community, and soil mineral properties when modeling soil C storage, particularly under global changes such as climate change and plant invasion.

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REFERENCES

Angst, G., Heinrich, L., Kogel-Knabner, I., & Mueller, C. W. (2016). The fate of cutin and suberin of decaying leaves, needles and roots – Inferences from the initial decomposition of bound fatty acids. *Organic Geochemistry*, *95*, 81–92.

Angst, G., Mueller, K. E., Kogel-Knabner, I., Freeman, K. H., & Mueller, C. W. (2017). Aggregation controls the stability of lignin and lipids in clay-sized particulate and mineral associated organic matter. *Biogeochemistry*, *132*, 307–324.

Austin, A. T., & Ballare, C. L. (2014). Plant interactions with other organisms: Molecules, ecology and evolution. *New Phytologist*, *204*, 257–260.

Baldock, J. A., Oades, J. M., Nelson, P. N., Skene, T. M., Golchin, A., & Clarke, P. (1997). Assessing the extent of decomposition of natural

organic materials using solid-state C-13 NMR spectroscopy. *Australian Journal of Soil Research*, *35*, 1061–1083.

Barney, J. N., Tharayil, N., Ditommaso, A., & Bhowmik, P. C. (2006). The biology of invasive alien plants in Canada. 5. *Polygonum cuspidatum* Sieb. & Zucc. [= Fallopia japonica (Houtt.) Ronse Decr.]. *Canadian Journal of Plant Science*, *86*, 887–905.

Belnap, J., Phillips, S. L., Sherrod, S. K., & Moldenke, A. (2005). Soil biota can change after exotic plant invasion: Does this affect ecosystem processes? *Ecology*, *86*, 3007–3017.

Blanco Canqui, H., & Lal, R. (2004). Mechanisms of carbon sequestration in soil aggregates. *Critical Reviews in Plant Sciences*, *23*, 481–504.

Callaway, R. M., Cipollini, D., Barto, K., Thelen, G. C., Hallett, S. G., Prati, D., … Klironomos, J. (2008). Novel weapons: Invasive plant suppresses fungal mutualists in America but not in its native Europe. *Ecology*, *89*, 1043–1055.

Callaway, R. M., Thelen, G. C., Rodriguez, A., & Holben, W. E. (2004). Soil biota and exotic plant invasion. *Nature*, *427*, 731–733.

Carpita, N. C. (1996). Structure and biogenesis of the cell walls of grasses. *Annual Review of Plant Physiology and Plant Molecular Biology*, *47*, 445–476.

Castellano, M. J., Mueller, K. E., Olk, D. C., Sawyer, J. E., & Six, J. (2015). Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. *Global Change Biology*, *21*, 3200–3209.

Craig, M. E., Pearson, S. M., & Fraterrigo, J. M. (2015). Grass invasion effects on forest soil carbon depend on landscape-level land-use patterns. *Ecology*, *96*, 2265–2279.

Clemente, J. S., Simpson, A. J., & Simpson, M. J. (2011). Association of specific organic matter compounds in size fractions of soils under different environmental controls. *Organic Geochemistry*, *42*, 1169–1180.

Clemmensen, K. E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., … Lindahl, B. D. (2013). Roots associated fungi drive long-term carbon sequestration in boreal forest. *Science*, *33*, 1615–1618.

Cotrufo, M. F., Soong, J. L., Horton, A. J., Campbell, E. E., Haddix, M. L., Wall, D. H., & Parton, A. J. (2015). Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nature Geoscience*, *8*, 776–781.

Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. (2013). The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? *Global Change Biology*, *19*, 988–995.

Courtier-Murias, D., Simpson, A. J., Marzadori, C., Baldoni, G., Ciavatta, C., Fernandez, J. M., … Plaza, C. (2013). Unraveling the long-term stabilization mechanisms of organic materials in soils by physical fractionation and NMR spectroscopy. *Agriculture Ecosystems & Environment*, *171*, 9–18.

Daynes, C. N., Field, D. J., Saleeba, J. A., Cole, M. A., & Mcgee, P. A. (2013). Development and stabilisation of soil structure via interactions between organic matter, arbuscular mycorrhizal fungi and plant roots. *Soil Biology & Biochemistry*, *57*, 683–694.

De Deyn, G. B., Cornelissen, J. H. C., & Bardgett, R. D. (2008). Plant functional traits and soil carbon sequestration in contrasting biomes. *Ecology Letters*, *11*, 516–531.

Derenne, S., & Largeau, C. (2001). A review of some important families of refractory macromolecules: Composition, origin, and fate in soils and sediments. *Soil Science*, *166*, 833–847.

Dixon J. B., & Weed S. B. (Eds.) (1989). *Soil Science Society of America book series, no. 1. Minerals in soil environments* (2nd ed., Xxvii+1244 p.). Madison, WI: Soil Science Society of America.

Dornbush, M. E. (2014). The myriad surprises of unwanted guests: Invasive plants and dynamic soil carbon pools. *New Phytologist*, *203*, 1–3. https://doi.org/10.1111/nph.12837

Drake, J. E., Gallet-Budynek, A., Hofmockel, K. S., Bernhardt, E. S., Billings, S. A., Jackson, R. B., … Finzi, A. C. (2011). Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO_2. *Ecology Letters*, *14*, 349–357.

Dungait, J. A. J., Hopkins, D. W., Gregory, A. S., & Whitmore, A. P. (2012). Soil organic matter turnover is governed by accessibility not recalcitrance. *Global Change Biology*, *18*, 1781–1796.

Elliott, E. T. (1986). Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. *Soil Science Society of America Journal*, *50*, 627–633.

Favilli, F., Egli, M., Cherubini, P., Sartori, G., Haeberli, W., & Delbos, E. (2008). Comparison of different methods of obtaining a resilient organic matter fraction in Alpine soils. *Geoderma*, *145*, 355–369.

Feng, X. J., Simpson, A. J., Wilson, K. P., Williams, D. D., & Simpson, M. J. (2008). Increased cuticular carbon sequestration and lignin oxidation in response to soil warming. *Nature Geoscience*, *1*, 836–839.

Fierer, N., Strickland, M. S., Liptzin, D., Bradford, M. A., & Cleveland, C. C. (2009). Global patterns in belowground communities. *Ecology Letters*, *12*, 1238–1249.

Filley, T. R., Boutton, T. W., Liao, J. D., Jastrow, J. D., & Gamblin, D. E. (2008). Chemical changes to nonaggregated particulate soil organic matter following grassland-to-woodland transition in a subtropical savanna. *Journal of Geophysical Research-Biogeosciences*, *113*, G03009.

Fontaine, S., Barot, S., Barre, P., Bdioui, N., Mary, B., & Rumpel, C. (2007). Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature*, *450*, 277–281.

Funk, J. L., & Vitousek, P. M. (2007). Resource-use efficiency and plant invasion in low-resource systems. *Nature*, *446*, 1079–1081.

Gaertner, M., Biggs, R., Te Beest, M., Hui, C., Molofsky, J., & Richardson, D. M. (2014). Invasive plants as drivers of regime shifts: Identifying high-priority invaders that alter feedback relationships. *Diversity and Distributions*, *20*, 733–744.

Gartner, T. B., & Cardon, Z. G. (2004). Decomposition dynamics in mixed-species leaf litter. *Oikos*, *104*, 230–246.

Geyer, K. M., Kyker-Snowman, E., Grandy, A. S., & Frey, S. D. (2016). Microbial carbon use efficiency: Accounting for population, community, and ecosystem-scale controls over the fate of metabolized organic matter. *Biogeochemistry*, *127*, 173–188.

Golchin, A., Baldock, J. A., & Oades, J. M. (1998). A model linking organic matter decomposition, chemistry, and aggregate dynamics. In R. Lal, J. M. Kimble, R. F. Follett & B. A. Stewart (Eds.), *Soil processes and the carbon cycle* (pp. 245–266). Boca Raton, FL: CRC Press.

Grandy, A. S., & Neff, J. C. (2008). Molecular C dynamics downstream: The biochemical decomposition sequence and its impact on soil organic matter structure and function. *Science of the Total Environment*, *404*, 297–307.

Haberhauer, G., & Gerzabek, M. H. (1999). Drift and transmission FT-IR spectroscopy of forest soils: An approach to determine decomposition processes of forest litter. *Vibrational Spectroscopy*, *19*, 413–417.

Hassink, J. (1997). The capacity of soils to preserve organic C and N by their association with clay and silt particles. *Plant and Soil*, *191*, 77–87.

Hatton, P. J., Castanha, C., Torn, M. S., & Bird, J. A. (2015). Litter type control on soil C and N stabilization dynamics in a temperate forest. *Global Change Biology*, *21*, 1358–1367.

Hickman, J. E., Wu, S. L., Mickley, L. J., & Lerdau, M. T. (2010). Kudzu (Pueraria montana) invasion doubles emissions of nitric oxide and increases ozone pollution. *Proceedings of the National Academy of Sciences of the United States of America*, *107*, 10115–10119.

Hobbs, R. J., Arico, S., Aronson, J., Baron, J. S., Bridgewater, P., Cramer, V. A., … Zobel, M. (2006). Novel ecosystems: Theoretical and management aspects of the new ecological world order. *Global Ecology and Biogeography*, *15*, 1–7.

Hobbs, R. J., Higgs, E., & Harris, J. A. (2009). Novel ecosystems: implications for conservation and restoration. *Trends in Ecology & Evolution*, *24*, 599–605.

Kaiser, K., & Guggenberger, G. (2000). The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. *Organic Geochemistry*, *31*, 711–725.

Kallenbach, C. M., Grandy, A. S., Frey, S. D., & Diefendorf, A. F. (2015). Microbial physiology and necromass regulate agricultural soil carbon accumulation. *Soil Biology & Biochemistry*, *91*, 279–290.

Keiluweit, M., Bougoure, J. J., Nico, P. S., Pett-Ridge, J., Weber, P. K., & Kleber, M. (2015). Mineral protection of soil carbon counteracted by root exudates. *Nature Climate Change*, *5*, 588–595.

Kleber, M., Mikutta, R., Torn, M. S., & Jahn, R. (2005). Poorly crystalline mineral phases protect organic matter in acid subsoil horizons. *European Journal of Soil Science*, *56*, 717–725.

Kleber, M., Sollins, P., & Sutton, R. (2007). A conceptual model of organo-mineral interactions in soils: Self-assembly of organic molecular fragments into zonal structures on mineral surfaces. *Biogeochemistry*, *85*, 9–24.

Kogel-Knabner, I. (2002). The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biology & Biochemistry*, *105*, A3–A8.

Kogel-Knabner, I. (2017). The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter: Fourteen years on. *Soil Biology & Biochemistry*, *34*, 139–162.

Kogel-Knabner, I., Guggenberger, G., Kleber, M., Kandeler, E., Kalbitz, K., Scheu, S., ... Leinweber, P. (2008). Organo-mineral associations in temperate soils: Integrating biology, mineralogy, and organic matter chemistry. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde*, *171*, 61–82.

Kramer, T. D., Warren, R. J. II., Tang, Y., & Bradford, M. A. (2012). Grass invasions across a regional gradient are associated with declines in belowground carbon pools. *Ecosystems*, *15*, 1271–1282.

Kulmatiski, A., Beard, K. H., Stevens, J. R., & Cobbold, S. M. (2008). Plant-soil feedbacks: A meta-analytical review. *Ecology Letters*, *11*, 980–992.

Kuzyakov, Y. (2010). Priming effects: Interactions between living and dead organic matter. *Soil Biology & Biochemistry*, *42*, 1363–1371.

Kuzyakov, Y., Friedel, J. K., & Stahr, K. (2000). Review of mechanisms and quantification of priming effects. *Soil Biology & Biochemistry*, *32*, 1485–1498.

Lajtha, K., Townsend, K. L., Kramer, M. G., Swanston, C., Bowden, R. D., & Nadelhoffer, K. (2014). Changes to particulate versus mineral-associated soil carbon after 50 years of litter manipulation in forest and prairie experimental ecosystems. *Biogeochemistry*, *119*, 341–360.

Lehmann, J., & Kleber, M. (2015). The contentious nature of soil organic matter. *Nature*, *528*, 60–68.

Leifeld, J. (2006). Application of diffuse reflectance FT-IR spectroscopy and partial least-squares regression to predict NMR properties of soil organic matter. *European Journal of Soil Science*, *57*, 846–857.

Levine, J. M., Adler, P. B., & Yelenik, S. G. (2004). A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters*, *7*, 975–989.

Liao, C. Z., Peng, R. H., Luo, Y. Q., Zhou, X. H., Wu, X. W., Fang, C. M., ... Li, B. (2008). Altered ecosystem carbon and nitrogen cycles by plant invasion: A meta-analysis. *New Phytologist*, *177*, 706–714.

Lindgren, C. J., Castro, K. L., Coiner, H. A., Nurse, R. E., & Darbyshire, S. J. (2013). The Biology of Invasive Alien Plants in Canada. 12. Pueraria montana var. lobata (Willd.) Sanjappa & Predeep. *Canadian Journal of Plant Science*, *93*, 71–95.

Lorenz, K., Lal, R., Preston, C. M., & Nierop, K. G. J. (2007). Strengthening the soil organic carbon pool by increasing contributions from recalcitrant aliphatic bio(macro)molecules. *Geoderma*, *142*, 1–10.

Macel, M., De Vos, R. C. H., Jansen, J. J., Van Der Putten, W. H., & Van Dam, N. M. (2014). Novel chemistry of invasive plants: Exotic species have more unique metabolomic profiles than native congeners. *Ecology and Evolution*, *4*, 2777–2786.

Mambelli, S., Bird, J. A., Gleixner, G., Dawson, T. E., & Torn, M. S. (2011). Relative contribution of foliar and fine root pine litter to the molecular composition of soil organic matter after in situ degradation. *Organic Geochemistry*, *42*, 1099–1108.

Mao, J. D., Hu, W. G., Schmidt-Rohr, K., Davies, G., Ghabbour, E. A., & Xing, B. S. (2000). Quantitative characterization of humic substances by solid-state carbon-13 nuclear magnetic resonance. *Soil Science Society of America Journal*, *64*, 873–884.

Martens, D. A., Reedy, T. E., & Lewis, D. T. (2004). Soil organic carbon content and composition of 130-year crop, pasture and forest land-use managements. *Global Change Biology*, *10*, 65–78.

Martin, P. A., Newton, A. C., & Bullock, J. M. (2017). Impacts of invasive plants on carbon pools depend on both species' traits and local climate. *Ecology*, *98*, 1026–1035.

Mueller, K. E., Eissenstat, D. M., Muller, C. W., Oleksyn, J., Reich, P. B., & Freeman, K. H. (2013). What controls the concentration of various aliphatic lipids in soil? *Soil Biology & Biochemistry*, *63*, 14–17.

Niemeyer, J., Chen, Y., & Bollag, J. M. (1992). Characterization of humic acids, composts, and peat by diffuse reflectance Fourier-transform infrared-spectroscopy. *Soil Science Society of America Journal*, *56*, 135–140.

O'Brien, S. L., & Jastrow, J. D. (2013). Physical and chemical protection in hierarchical soil aggregates regulates soil carbon and nitrogen recovery in restored perennial grasslands. *Soil Biology & Biochemistry*, *61*, 1–13.

Otto, A., Shunthirasingham, C., & Simpson, M. J. (2005). A comparison of plant and microbial biomarkers in grassland soils from the Prairie Ecozone of Canada. *Organic Geochemistry*, *36*, 425–448.

Otto, A., & Simpson, M. J. (2007). Analysis of soil organic matter biomarkers by sequential chemical degradation and gas chromatography – Mass spectrometry. *Journal of Separation Science*, *30*, 272–282.

Paterson, E., Osler, G., Dawson, L. A., Gebbing, T., Sim, A., & Ord, B. (2008). Labile and recalcitrant plant fractions are utilised by distinct microbial communities in soil: Independent of the presence of roots and mycorrhizal fungi. *Soil Biology & Biochemistry*, *40*, 1103–1113.

Peltzer, D. A., Allen, R. B., Lovett, G. M., Whitehead, D., & Wardle, D. A. (2010). Effects of biological invasions on forest carbon sequestration. *Global Change Biology*, *16*, 732–746.

Peltzer, D. A., Bellingham, P. J., Kurokawa, H., Walker, L. R., Wardle, D. A., & Yeates, G. W. (2009). Punching above their weight: Low-biomass non-native plant species alter soil properties during primary succession. *Oikos*, *118*, 1001–1014.

Penuelas, J., Sardans, J., Llusia, J., Owen, S. M., Silva, J., & Niinemets, U. (2010). Higher allocation to low cost chemical defenses in invasive species of Hawaii. *Journal of Chemical Ecology*, *36*, 1255–1270.

Phillips, R. P., Finzi, A. C., & Bernhardt, E. S. (2011). Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. *Ecology Letters*, *14*, 187–194.

Post, W. M., Emanuel, W. R., Zinke, P. J., & Stangenberger, A. G. (1982). Soil carbon pools and world life zones. *Nature*, *298*, 156–159.

Prescott, C. E. (2010). Litter decomposition: What controls it and how can we alter it to sequester more carbon in forest soils? *Biogeochemistry*, *101*, 133–149.

Preston, C. M., Trofymow, J. A., & The Canadian Intersite Decomposition Experiment Working Group. (2000). Variability in litter quality and its relationship to litter decay in Canadian forests. *Canadian Journal of Botany-Revue Canadienne De Botanique*, *78*, 1269–1287.

Rasse, D. P., Rumpel, C., & Dignac, M. F. (2005). Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant and Soil*, *269*, 341–356.

Ridenour, W. M., Vivanco, J. M., Feng, Y. L., Horiuchi, J., & Callaway, R. M. (2008). No evidence for trade-offs: Centaurea plants from

America are better competitors and defenders. *Ecological Monographs*, *78*, 369–386.

Rillig, M. C., & Mummey, D. L. (2006). Mycorrhizas and soil structure. *New Phytologist*, *171*, 41–53.

Sax, D. F., Stachowicz, J. J., Brown, J. H., Bruno, J. F., Dawson, M. N., Gaines, S. D., … Rice, W. R. (2007). Ecological and evolutionary insights from species invasions. *Trends in Ecology & Evolution*, *22*, 465–471.

Schlesinger, W. H. (1977). Carbon balance in terrestrial detritus. *Annual Review of Ecology and Systematics*, *8*, 51–81.

Schmidt, M. W. I., Knicker, H., Hatcher, P. G., & Kogel-Knabner, I. (1997). Improvement of 13C and 15N CPMAS NMR spectra of bulk soils, particle size fractions and organic material by treatment with 10% hydrofluoric acid. *European Journal of Soil Science*, *48*, 319–328.

Schmidt, M. W. I., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., … Trumbore, S. E. (2011). Persistence of soil organic matter as an ecosystem property. *Nature*, *478*, 49–56.

Seastedt, T. R., Hobbs, R. J., & Suding, K. N. (2008). Management of novel ecosystems: Are novel approaches required? *Frontiers in Ecology and the Environment*, *6*, 547–553.

Simpson, M. J., & Simpson, A. J. (2012). The chemical ecology of soil organic matter molecular constituents. *Journal of Chemical Ecology*, *38*, 768–784.

Six, J., Conant, R. T., Paul, E. A., & Paustian, K. (2002). Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. *Plant and Soil*, *241*, 155–176.

Six, J., Paustian, K., Elliott, E. T., & Combrink, C. (2000). Soil structure and organic matter: I. Distribution of aggregate-size classes and aggregate-associated carbon. *Soil Science Society America Journal*, *64*, 681–689.

Sjogersten, S., Turner, B. L., Mahieu, N., Condron, L. M., & Wookey, P. A. (2003). Soil organic matter biochemistry and potential susceptibility to climatic change across the forest-tundra ecotone in the Fennoscandian mountains. *Global Change Biology*, *9*, 759–772.

Strauss, S. Y., Webb, C. O., & Salamin, N. (2006). Exotic taxa less related to native species are more invasive. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 5841–5845.

Strickland, M. S., Devore, J. L., Maerz, J. C., & Bradford, M. A. (2010). Grass invasion of a hardwood forest is associated with declines in belowground carbon pools. *Global Change Biology*, *16*, 1338–1350.

Suseela, V., Alpert, P., Nakatsu, C. H., Armstrong, A. & Tharayil, N. (2016). Plant–soil interactions regulate the identity of soil carbon in invaded ecosystems: Implication for legacy effects. *Functional Ecology*, *30*, 1227–1238.

Suseela, V., Tharayil, N., Xing, B. S., & Dukes, J. S. (2013). Labile compounds in plant litter reduce the sensitivity of decomposition to warming and altered precipitation. *New Phytologist*, *200*, 122–133.

Suseela, V., Tharayil, N., Xing, B. S., & Dukes, J. S. (2014). Warming alters potential enzyme activity but precipitation regulates chemical transformations in grass litter exposed to simulated climatic changes. *Soil Biology & Biochemistry*, *75*, 102–112.

Tamura, M., & Tharayil, N. (2014). Plant litter chemistry and microbial priming regulate the accrual, composition and stability of soil carbon in invaded ecosystems. *New Phytologist*, *203*, 110–124.

Tharayil, N., Alpert, P., Bhowmik, P., & Gerard, P. (2013). Phenolic inputs by invasive species could impart seasonal variations in nitrogen pools in the introduced soils: A case study with *Polygonum cuspidatum*. *Soil Biology & Biochemistry*, *57*, 858–867.

Tharayil, N., Bhowmik, P., Alpert, P., Walker, E., Amarasiriwardena, D., & Xing, B. (2009). Dual purpose secondary compounds: Phytotoxins of *Centaurea diffusa* also facilitates nutrient uptake. *New Phytologist*, *181*, 424–434.

Tharayil, N., Suseela, V., Preston, C. M., Triebwasser, J. D., Dukes, J. S., & Gerard, P. (2011). Changes in the structural composition and reactivity of Acer rubrum leaf litter tannins exposed to warming and altered precipitation: climatic-stress-induced tannins are more reactive. *New Phytologist*, *191*, 132–145.

Torn, M. S., Swanston, C. W., Castanha, C., & Trumbore, S. E. (2009). Storage and turnover of organic matter in soil. In N. Senesi, B. Xing & P. M. Huang (Eds.), *Biophysico-chemical processes involving natural nonliving organic matter in environmental systems* (pp. 219–272). Hoboken, NJ: John Wiley & Sons, Inc.

Van Kleunen, M., Weber, E., & Fischer, M. (2010). A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters*, *13*, 235–245.

Van Meerbeek, K., Appels, L., Dewil, R., Calmeyn, A., Lemmens, P., Muys, B., & Hermy, M. (2015). Biomass of invasive plant species as a potential feedstock for bioenergy production. *Biofuels, Bioproducts and Biorefining*, *9*, 273–282.

Vitousek, P. M. (1990). Biological invasions ecosystem process: Towards an integration of population biology ecosystem studies. *Oikos*, *57*, 7–13.

Von Lutzow, M., Kogel-Knabner, I., Ekschmitt, K., MFfigatzner, E., Guggenberger, G., Marschner, B., & Flessa, H. (2006). Stabilization of organic matter in temperate soils: Mechanisms and their relevance under different soil conditions – A review. *European Journal of Soil Science*, *57*, 426–445.

Voriskova, J., Brabcova, V., Cajthaml, T., & Baldrian, P. (2014). Seasonal dynamics of fungal communities in a temperate oak forest soil. *New Phytologist*, *201*, 269–278.

Wang, J., Tharayil, N., Chow, A. T., Suseela, V., & Zeng, H. (2015). Phenolic profile within the fine root branching orders of an evergreen species highlights a disconnect in root tissue quality predicted by elemental- and molecular-level carbon composition. *New Phytologist*, *206*, 1261–1273.

Wardle, D. A., Yeates, G. W., Barker, G. M., & Bonner, K. I. (2006). The influence of plant litter diversity on decomposer abundance and diversity. *Soil Biology & Biochemistry*, *38*, 1052–1062.

Winkler, A., Haumaier, L., & Zech, W. (2005). Insoluble alkyl carbon components in soils derive mainly from cutin and suberin. *Organic Geochemistry*, *36*, 519–529.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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