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Plant litter chemistry and microbial priming regulate the accrual, composition and stability of soil carbon in invaded ecosystems

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Summary

- Soil carbon (C) sequestration, as an ecosystem property, may be strongly influenced by invasive plants capable of depositing disproportionately high quantities of chemically distinct litter that disrupt ecosystem processes. However, a mechanistic understanding of the processes that regulate soil C storage in invaded ecosystems remains surprisingly elusive.
- Here, we studied the impact of the invasion of two noxious nonnative species, *Polygonum cuspidatum*, which produces recalcitrant litter, and *Pueraria lobata*, which produces labile litter, on the quantity, molecular composition, and stability of C in the soils they invade.
- Compared with an adjacent noninvaded old-field, *P. cuspidatum*-invaded soils exhibited a 26% increase in C, partially through selective preservation of plant polymers. Despite receiving a 22% higher litter input, *P. lobata*-invaded *Pinus* stands exhibited a 28% decrease in soil C and a twofold decrease in plant biomarkers, indicating microbial priming of native soil C. The stability of C exhibited an opposite trend: the proportion of C that was resistant to oxidation was 21% lower in *P. cuspidatum*-invaded soils and 50% higher in *P. lobata*-invaded soils.
- Our results highlight the capacity of invasive plants to feed back to climate change by destabilizing native soil C stocks and indicate that environments that promote the biochemical decomposition of plant litter would enhance the long-term storage of soil C. Further, our study highlights the concurrent influence of dominant plant species on both selective preservation and humification of soil organic matter.

Introduction

Invasion by exotic plants and climate change are two major environmental changes that may threaten the sustainability of global ecosystems. Exotic plant species now account for *c.* 33% of the total flora in the British Isles and invade *c.* 700 000 ha of US wildlife habitat annually (Pimentel, 2002). This results in an annual economic cost that exceeds $34 billion and threatens the biodiversity of native ecosystems (Pimentel *et al.*, 2005; Gilbert & Levine, 2013). Similarly, the anticipated increase in average global temperature not only challenges the survival of many species but also accelerates the loss of stored carbon (C) from soils, which can spur climate change through the greenhouse effect (Davidson & Janssens, 2006; Conant *et al.*, 2011). The extent of this detrimental positive feedback of warming on soil C loss is determined by the stability of soil organic matter (SOM; Davidson & Janssens, 2006), which in turn is dependent upon the processes leading to its formation (Trumbore & Czimczik, 2008; Prescott, 2010; Schmidt *et al.*, 2011). The above two global changes are predicted to interact catastrophically, with climate change facilitating a massive range expansion of many exotic species by the turn of this century (Dukes & Mooney, 1999; Bradley *et al.*, 2010; Dawson *et al.*, 2012). However, the potential for plant invasions to participate in feedback loops that affect climate change by regulating the accrual, composition, and stability of soil organic matter (SOM) in invaded ecosystems is remarkably less studied (Fig. 1a).

The C sequestration potential of ecosystems is predominantly determined by the balance between the reduction of atmospheric C by primary producers during photosynthesis and the subsequent release of the photosynthetically fixed C back to the atmosphere through oxidation (respiration and combustion). Across most terrestrial ecosystems, the C fixed through photosynthesis serves as the source of organic substrates that fuel heterotrophic metabolism (Kogel-Knabner, 2002; Trumbore & Czimczik, 2008; Prescott, 2010). Thus, the physical and physiological traits of the primary producers that regulate the initial C assimilation could directly and indirectly influence the abundance and metabolism of soil biota that facilitate SOM formation (De Deyn *et al.*, 2008). For example, plants adapted to nutrient-poor environments invest more in defence compounds during growth, and resorb nutrients from leaves during senescence in an effort to protect the already acquired resources (Herms & Mattson, 1992), thus producing nutrient-poor recalcitrant litter. This recalcitrant litter would in turn promote the fungal communities that are specialized in degrading complex plant polymers. The slow rate of

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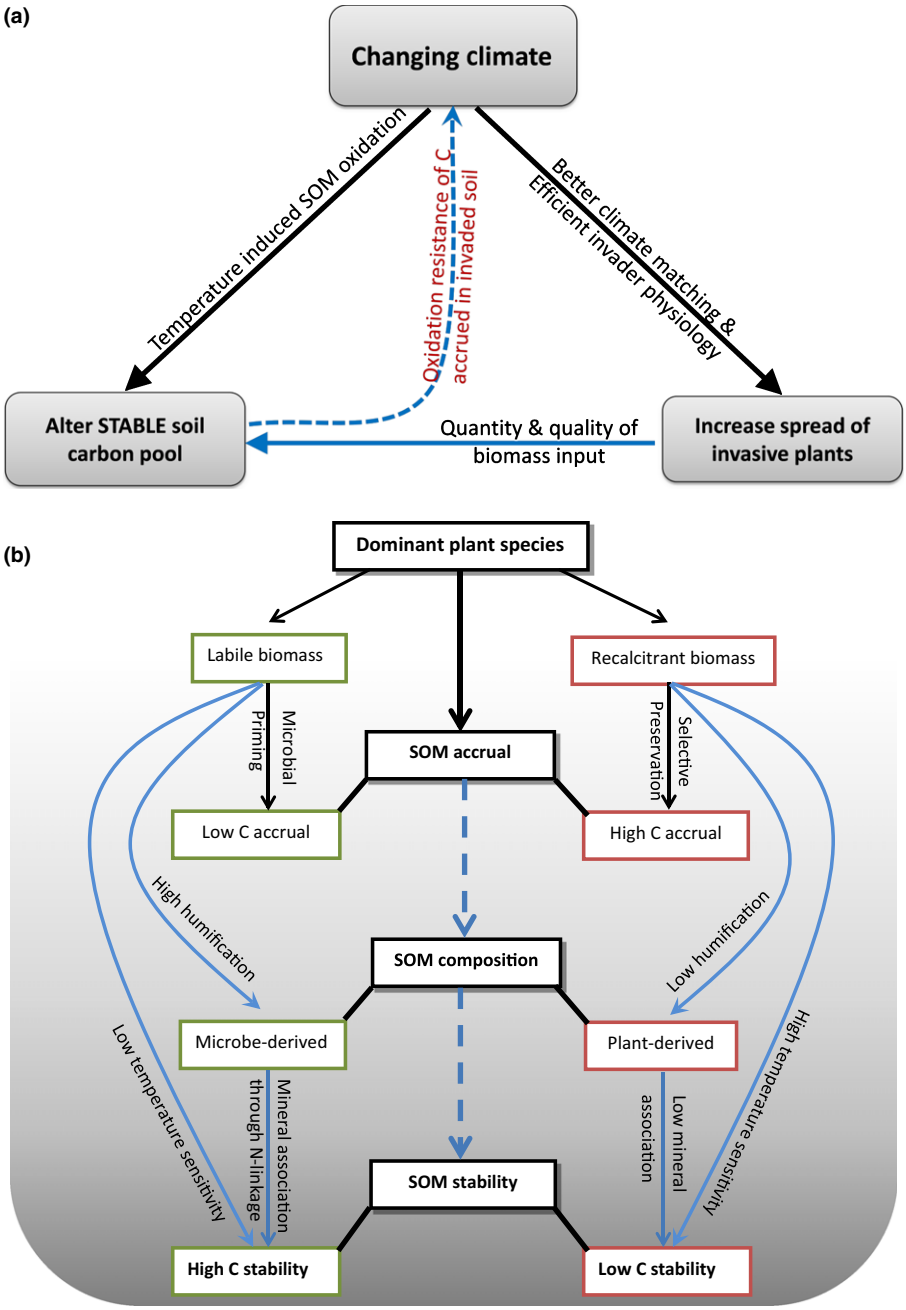


Fig. 1 (a) Schematic diagram outlining the scientific question addressed in the present study in the context of current knowledge. Black arrows represent well-studied relationships, whereas solid and broken blue arrows represent less known mechanisms. Climate warming is projected to accelerate the oxidation of soil carbon (C) stocks, thus creating a positive feedback to climate change (Davidson & Janssens, 2006). Invasive plant species are predicted to undergo massive range expansion under projected climates as a result of better climate matching (Bradley *et al.*, 2010) and physiological adaptability of the invader to changing climates (Sorte *et al.*, 2013). Though invasive plants are reported to alter soil C storage in the invaded ecosystems (Liao *et al.*, 2008; Peltzer *et al.*, 2010), a mechanistic understanding of the factors driving this change remains surprisingly unexplored. Equally little studied is the ability of invasive species to feed back to climate change by altering the oxidation susceptibility of the C stocks in invaded ecosystems. Thus, the proposed study captures the potential fate of soil C in the confluence of two global changes: plant invasion and climate change. (b) Schematic diagram representing the current knowledge (nonshaded area) linking plant litter input to soil organic matter (SOM) accrual, and the proposed mechanisms mediated by litter quality, microbial processes, and mineral associations that differentially link SOM accrual to its composition and stability (increasing intensity of grey, blue arrows and the broken arrow represent less known mechanisms). Current paradigms positively relate plant input to soil C sequestration, with litter chemistry regulating the extent of sequestration: low-quality (recalcitrant) plant inputs lead to higher storage of soil C potentially as a result of the selective preservation of plant biopolymers, whereas high-quality (labile) plant inputs lead to a lower C storage as a result of faster C cycling (Fontaine *et al.*, 2007; De Deyn *et al.*, 2008). As most of the humified materials are thought to be capable of forming strong mineral associations (Cotrufo *et al.*, 2013), possibly through nitrogen linkages to mineral surfaces (Kleber *et al.*, 2007), the SOM formed from labile plant inputs, though low in quantity, could be less susceptible to warming-induced oxidation (low temperature sensitivity). However, the SOM formed through selective preservation will be less mineral-associated (Conant *et al.*, 2011) and more prone to oxidation under warmer climates.

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fungal metabolism would impede the nutrient cycling in these nutrient-poor ecosystems to match the rate of nutrient release to the rate of nutrient uptake by slow-growing plant species (Hattenschwiler & Vitousek, 2000). This coordinated nutrient cycling, while retaining the resources within these ecosystems, also increases the residence time of assimilated C. Thus, along with the edaphic factors, the identity of the dominant plant species could regulate the potential for soil C sequestration in natural ecosystems (Makkonen *et al.*, 2012).

However, despite this potential ability of the plants to modulate soil C storage (De Deyn *et al.*, 2008; Makkonen *et al.*, 2012), the direct effect of plant inputs on SOM accrual and composition is highly debated (Marschner *et al.*, 2008; Dungait *et al.*, 2012; Stark *et al.*, 2012). Plant heteropolymers such as lignins, tannins and cuticular matrices are slow to decompose (Lorenz *et al.*, 2007; Suseela *et al.*, 2013). Thus, ecosystems that produce biomass abundant in recalcitrant compounds are thought to facilitate a higher sequestration of atmospheric CO_2 in soils (selective preservation; Lichtfouse *et al.*, 1998; Lorenz *et al.*, 2007). Supporting this notion is the observation that chemically complex plant-derived compounds are found to be selectively preserved in the SOM fractions with intermediate to slow turnover times in terrestrial (Bol *et al.*, 1996; Filley *et al.*, 2008; Crow *et al.*, 2009; Feng *et al.*, 2010; Stewart *et al.*, 2011; Stark *et al.*, 2012; Mueller *et al.*, 2013) and marine (Hedges *et al.*, 2001; Huguet *et al.*, 2008) ecosystems, which have been successfully used to infer past vegetation changes in paleoecological studies (Jansen *et al.*, 2006; Nierop & Jansen, 2009; Pautler *et al.*, 2013; Pisani *et al.*, 2013). However, recent studies have conclusively identified the microbial-mediated decomposition and resynthesis of plant inputs as the key process shaping stable soil C stocks (Schmidt *et al.*, 2011), resulting in plant litter having a rather fleeting influence on the amount and composition of SOM (Gentile *et al.*, 2011; Mambelli *et al.*, 2011).

Similar to the above contention regarding the influence of recalcitrant plant inputs on the quantity and composition of SOM, the influence of labile plant inputs (low in C : nitrogen (N) and heteropolymers) on the oxidation resistance (stability) of SOM still evades conceptual understanding. The current paradigm dictates that the input of easily degradable plant metabolites would destabilize soil C stocks by facilitating the microbial co-metabolism of recalcitrant compounds, resulting in a net loss of C from soils (positive priming effect; Fontaine *et al.*, 2007). However, humified SOM that is formed through extensive biochemical and abiotic decomposition of plant inputs (humification; Guggenberger, 2005) is hypothesized to exhibit a higher mineral association (Clemente & Simpson, 2013; Cotrufo *et al.*, 2013), indicating that microbial decomposition may offer better stability to SOM. Thus, humification could potentially promote the build-up of stable soil C pools (Prescott, 2010). The above contradiction highlights the current disconnect between the litter decomposition processes and the SOM formation pathways. This knowledge gap further increases the uncertainty in forecasting the potential of plant invasions to exacerbate climate change by destabilizing soil C stocks.

Considering that only a minute fraction of the plant input finds its way into the final SOM pool (Berg & McClaugherty, 2003), the above discrepancy in the role of plant litter chemistry in SOM formation may be attributable to the dilution of the impact of new organic matter that was formed in the larger pool of resident SOM. Because SOM formation is an ecosystem property (De Deyn *et al.*, 2008; Schmidt *et al.*, 2011) and ecosystems develop and flourish over decadal time-scales, the influence of plant identity on SOM characteristics could be better captured in invaded ecosystems where an exotic species dominates and regulates ecosystem processes for prolonged time periods (Vitousek, 1990; Sax *et al.*, 2007).

In general, the mechanisms of invasion exhibited by most invasive plant species could be summarized as strategies that would facilitate better resource competition. Most invasive plants have higher resource acquisition (Tharayil *et al.*, 2009) and resource-use efficiencies (Funk & Vitousek, 2007). Thus, the majority of the invasive plant species produce a higher biomass compared with the natives that they displace (Knapp *et al.*, 2008; Liao *et al.*, 2008; Laungani & Knops, 2009; Ehrenfeld, 2010). Similarly, most invasive plants often exhibit litter chemistries that are distinct from those of native plant species (Cappuccino & Arnason, 2006), as these novel chemistries confer a greater protection to exotic species from native herbivores (Weidenhamer & Callaway, 2010; Pintó-Marijuan & Munné-Bosch, 2013). These chemically distinct inputs also alter the nutrient cycling and heterotrophic community composition of the invaded soils (Ehrenfeld, 2010), resulting in the formation of novel ecosystems that are stable and resilient to further changes (Hobbs *et al.*, 2006; Seastedt *et al.*, 2008). Thus, it could be postulated that invasive plants that input disproportionate quantities of chemically distinct litter could exert an undue influence on the accrual and stability of SOM in ecosystems they invade.

Despite a higher net primary productivity, recent meta-analyses have documented contrasting influences of invasive plants on SOM accrual (Liao *et al.*, 2008; Eldridge *et al.*, 2011). While a majority of the studies have reported an increase in soil C stocks of invaded ecosystems (Knapp *et al.*, 2008; Wolkovich *et al.*, 2010), others have documented a decline in soil C concentrations following the invasion of exotic species (Jackson *et al.*, 2002; Koteen *et al.*, 2011; Kramer *et al.*, 2012; Shang *et al.*, 2013). For example, encroachment of an invasive shrub *Prosopis velutina* increased the soil organic C of native grasslands by *c.* 155% (Wheeler *et al.*, 2007), and the invasion of a C_4 grass *Microstegium vimineum* into native forests decreased the soil organic C and particulate organic matter by 24% and 34%, respectively (Strickland *et al.*, 2010). Despite this stark contrast in the influence of exotic invasive plants on soil C sequestration, a mechanistic understanding of the processes driving these responses remains surprisingly unexplored. Similarly, the stability of the newly formed soil C in the invaded landscapes has never been investigated. The stability of the sequestered SOM is more important for the effective storage of C under future warmer climates (Prescott, 2010), hence the capacity of high-biomass-producing exotic plants to increase the stable soil C pool could be misleading. A better understanding of the influence of invasive species on soil C stabilization would not only help to predict the fate of soil C in invaded ecosystems under future warmer climates

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but would also provide a unique opportunity to document the long-term role of litter chemistry in the formation and stabilization of SOM in natural environments, which could inform soil C management strategies.

We hypothesized that the SOM formation processes, and hence the total quantity, composition, and, more importantly, the stability of soil C, will diverge between invaded and adjacent noninvaded soils in ecosystems subjected to prolonged invasion by exotic plants with contrasting litter chemistries (recalcitrant versus labile; Fig. 1b). We predicted that, compared with their respective adjacent noninvaded soils inhabited by native plant species, (1) the soils invaded by exotic species that produce recalcitrant litter will exhibit an increase in the total soil C, whereas invasion by a species that produces labile litter will result in a decrease in the native soil C stocks; (2) the organic matter in soils invaded by species that input recalcitrant litter will be characterized by a higher proportion of plant-derived biomarkers, indicating selective preservation of plant polymers, whereas SOM associated with the invader that produces labile litter will be depleted in plant-derived biomarkers, indicating an accelerated humification; and (3) although high in quantity, the soil C accrued under the invader that produces recalcitrant litter will be more susceptible to oxidation, whereas the proportion of oxidation-resistant C will be higher in soils invaded by species that produce labile litter.

To understand the effects of plant invasions and the associated litter chemistry on the accrual, composition and stabilization of soil C, we selected two perennial invasive species with distinct litter quality (Notes S1): *Polygonum cuspidatum* Siebold & Zucc. (Japanese knotweed), which produces polyphenol-rich recalcitrant litter (Suseela *et al.*, 2013; Tharayil *et al.*, 2013; Table 1), and leguminous *Pueraria montana* var. *lobata* (Willd.) Sanjappa & Predeep (kudzu), which produces N-rich labile litter (N-fixation: 235 kg N ha^{-1} yr^{-1}; Hickman *et al.*, 2010; Lindgren *et al.*, 2013; Table 2, Supporting Information Fig. S1). Both of the study species invade a wide range of edaphic conditions, and climate is the major factor regulating the spread of these two exotic species in North America (Barney *et al.*, 2006; Lindgren *et al.*, 2013; Notes S1). The influence of litter chemistry in SOM formation was further emphasized by selecting study sites where the above invasive species were encroaching into ecosystems with contrasting resident litter chemistries. Knotweed was invading an old-field ecosystem that produced relatively labile litter, and kudzu was invading *Pinus* forest that input recalcitrant litter (Tables 1, 2).

Materials and Methods

Study site and soil sampling

Knotweed is a federally noxious weed in many countries across Europe and is present in 35 states in the USA, in five of which it is classified as a noxious weed (Tharayil *et al.*, 2013). Kudzu is a noxious weed in the southeastern USA, has invaded > 3 million ha of forest and is spreading at a rate of 50 000 ha yr^{-1} (Hickman *et al.*, 2010), resulting in an estimated loss of $336 million annually in forest production (Lindgren *et al.*, 2013).

Study sites were selected close to the point of initial introduction of these two species in North America. Knotweed invasion was studied in *c.* 15 ha of an old-field in Amherst, Massachusetts that had been traditionally under alfalfa (*Medicago sativa*) cultivation until its abandonment during the period 1990–1992 (42°24′N, 72°31′W). Kudzu invasion was studied in a *c.* 20-ha *Pinus* forest in Seneca, South Carolina (34°41′N, 82°53′W), where kudzu was completely covering the ground and partially climbing over established *Pinus taeda* stands. At both sites, the invasive species represented an established population that was at least 20 yr old, and the progression of invasion at these sites has been recorded since 2002 (Barney *et al.*, 2006; Tharayil *et al.*, 2013). Understory vegetation was completely absent under knotweed stands and was very sparse under kudzu and *Pinus* stands throughout the growing season. The noninvaded plots bordering knotweed invasion were dominated by a typical old-field community consisting of both grasses and forbs (*Aesculus* sp., *Dactylis* sp., *Elytrigia repens*, *Galium* sp.,

Table 1 Characteristics of soils[1] and litter[2] input in the *Polygonum cuspidatum* invaded site[3]

	0–5 cm		5–10 cm		10–15 cm	
	Invaded	Noninvaded	Invaded	Noninvaded	Invaded	Noninvaded
Soil pH	5.7 (0.3)^a	5.9 (0.6)^a	5.8 (0.3)^a	6.0 (0.6)^a	6.1 (0.4)^a	6.0 (0.4)^a
Sand (%)	48.7 (1)^a	50.2 (1)^a	47.0 (0.8)^a	47.2 (0.6)^a	45.2 (1.3)^a	44.1 (1.6)^a
Silt (%)	44.5 (0.6)^a	45.2 (0.9)^a	46.7 (0.6)^a	46.9 (0.4)^a	50.2 (1.4)^a	51.1 (1.7)^a
Clay (%)	5.8 (0.2)^a	5.9 (0.3)^a	5.5 (0.4)^a	5.5 (0.2)^a	5.9 (0.6)^a	6 (0.2)^a
Bulk density (Mg m^{-3})	1.06 (0.07)^a	1.14 (0.09)^a	1.21 (0.07)^a	1.16 (0.08)^a	1.33 (0.06)^a	1.36 (0.11)^a
Soil N (Mg ha^{-1})	176 (34)^a	145 (100)^b	86 (5)^a	94 (11)^a	78 (7)^a	91 (5)^a
Root biomass (g m^{-2})	141 (22)^a	165 (24)^a	98 (22)^b	181 (36)^a	102 (27)^b	158 (31)^a
Shoot biomass (g m^{-2})	1166 (185)^a	359 (95)^b				
Biomass C : N	128 (15)^a	41 (18)^b				
Biomass lignin (%)	7.5 (1.2)^a	1.9 (0.5)^b				
Biomass tannin (%)	10.4 (2.1)	Not detected				
Biomass lipids (%)	0.8 (0.2)^a	0.4 (0.2)^b				

[1]Agawam fine sandy loam soil (mesic Typic Dystrochrepts).
[2]Litter input was measured by harvesting above-ground biomass in a 0.5-m^2 area within the sampling plots.
[3]Values represent mean and standard deviation. Parameters within the same depth were compared with one-way ANOVA; significant differences ($P < 0.05$) between values within a depth are represented by differing superscript letters.

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Lepidium spp., *Oxalis stricta*, *Plantago* spp., *Rhus glabra*, *Schedonorus phoenix*, *Setaria pumila*, *Trifolium* sp., and *Vicia* sp.), and the leguminous N-fixing forbs contributed 35 ± 9% of the biomass in these noninvaded plots. The growing season of both knotweed and the adjacent old-field species extends from April to October, after which the above-ground biomass senesces, whereas kudzu invasion into evergreen *Pinus* forest is seasonal: kudzu emerges during early summer in June and senesces upon exposure to the first frost during October–November.

Six spatially separated monospecific stands of the invasive species were randomly located at each site. These stands served as independent experimental units within each invaded site. Within each stand, three parallel transects were run perpendicular to the edge of invasion at 6–8-m intervals, and a 1-m^2 sampling plot was marked inside (at least 8 m inside the edge of the invasion; IN) and outside (3–4 m outside the invasion edge; OUT) the invasion along each transect. The use of paired invaded and adjacent noninvaded plots that are similar in soil physical characteristics to study the ecosystem-level impacts of plant invasion is in accordance with the conventional approach adopted by most studies in invasion ecology (Jackson *et al.*, 2002; Filley *et al.*, 2008; Strickland *et al.*, 2010). This approach meaningfully delineates the direct impact of the invader on ecosystem processes from any effects contributed by the inherent variation in edaphic and climatic conditions. In July 2011, the surface organic layer was carefully removed and three soil cores of 10 cm diameter were collected from the underlying mineral layer to a depth of 15 cm at 5-cm intervals from each sampling plot. Samples from the same depth at each sampling plot were combined, mixed, placed in a plastic bag on ice, and transported to the lab, where the samples were further homogenized and sieved through 2-mm mesh. Any recognizable root materials were separated, and the soil samples were stored at 2–4°C until analyses.

Above-ground litter from the knotweed site was harvested from sampling plots at the end of the growing season. The annual above-ground litter input in kudzu-invaded sites was calculated from the fresh litter input in randomly selected 0.5-m^2 plots. The litter was collected soon after senescence and stored at 4°C until analysis. The root biomass at both study sites was estimated from the roots retrieved from the soil cores.

Chemical analyses

Chemical characterization of plant litter The biopolymer composition (plant sterols, lignins, and lipids that represent cutin, waxes and suberins) of the freshly senesced plant litter was characterized following sequential solvent extraction, base hydrolysis and copper oxide (CuO) oxidation using the biomarker approach described in the soil characterization section (Biomarker analyses to assess the composition of SOM) below. Tannins in the senesced tissues were quantified by an acid-butanol assay as described in Tharayil *et al.* (2011) using the tannins purified from the respective species.

Soil microbial biomass estimation Microbial biomass in soils was assessed by quantifying the ergosterol and muramic acid content. Ergosterol, an indicator of fungal biomass, was extracted from soils following base hydrolysis as per Montgomery *et al.* (2000), and muramic acid, an indicator of bacterial biomass, was extracted from soils after acid hydrolysis as per Joergensen & Wichern (2008), and analysed using a high-pressure liquid chromatograph (HPLC). Ergosterol was quantified using absorbance at 282 nm, and muramic acid was quantified after derivatization with ortho-phthaldialdehyde using a fluorescence detector (excitation and emission wavelengths = 340 and 455 nm, respectively; see Methods S1 for detailed methodology).

Biomarker analyses to assess the composition of SOM The biomarker approach is focused on the extraction and molecular-level identification of different biomolecules from SOM. The biomarker approach provides compound-specific information of the extractable fractions of SOM, and facilitates the robust classification of extracted compounds to plant/microbial source (Otto *et al.*, 2005), which has been verified using carbon-13 nuclear magnetic resonance (NMR) spectroscopy (Simpson *et al.*, 2008).

Solvent extraction of free lipids Briefly, soil and litter samples underwent sequential extraction with methanol, methanol : dichlolomethane (1 : 1; v/v), and dichloromethane, and the supernatants were combined and concentrated using a rotary evaporator and further dried completely under N gas. The major components in the solvent extract of SOM include carbohydrates, alkanes, alkanols, alkanoic acids and sterols that indicate the presence of plant waxes (long-chain alkanoic acids and alkanols) and microbial tissues (branched short-chain alkanoic acids and ergosterol).

Base hydrolysis of bound lipids Following solvent extraction, the soils were further subjected to base hydrolysis for the chemolytic cleavage and extraction of the ester-bound lipids. The base hydrolysates consist predominantly of hydroxy-acids, mid-chain substituted acids and phenols that represent plant polymers including cutin, suberin, lignins and waxes. Briefly, a subsample of solvent-extracted soil was base-hydrolysed by refluxing with 1N methanolic KOH to release ester-bound lipids. Hydrolysable lipids were recovered using liquid–liquid extraction with diethyl ether and dried under N gas.

CuO oxidation The soils were further oxidized in the presence of CuO to depolymerize the lignin residues in SOM. Briefly, the base-extracted soil was combined with CuO, ferrous ammonium sulfate, and 2 M NaOH in a Teflon-lined acid digestion vessel and heated to 160°C for 160 min. The supernatant was extracted with ethyl acetate, and the ethyl acetate fraction was collected and stored at −20°C for further analysis (see Methods S1 for detailed methodology on biomarker analyses).

GC-MS conditions for biomarker analysis For the GC-MS analysis, an aliquot of the samples obtained from the above extractions was silylated with 200 µl of *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) with 1% trimethylchlorosilane (TMCS) at 50°C for 60 min. The silylated samples were analysed using an Agilent 7980A GC system coupled to a 5975 C Series

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mass detector (Agilent Technologies, Santa Clara, CA, USA). The separation of the compounds was achieved on a DB-5 MS fused-silica capillary column (30 m length × 0.25 mm internal diameter × 0.20 μm film thickness; Agilent Technologies) using a split (1 : 10) injection. Compounds were positively identified based on comparison of the mass fragmentation pattern with Wiley 9th + NIST08 MS Libraries (Agilent Technologies), and comparison with external standards and the literature (Otto *et al.*, 2005). Compounds were quantified based on external calibration curves using authentic standards for phenolic compounds, plant sterols, alkanes, and methylated and silylated lipids. Monomer identification of cutin and waxes was based on depolymerization of purified cuticle from tomato (*Lycopersicon esculentum*), apple (*Malus domestica*; fruit cuticle), and knotweed (stems), and identification of suberin monomers was based on depolymerization of suberin from tubers of potato (*Solanum tuberosum*) and sweet potato (*Ipomoea batatas*) and roots of knotweed, grasses, and mixed kudzu and *Pinus* (Järvinen *et al.*, 2009). For compounds without authentic standards, the quantifications were based on proxy compounds; alkanols were quantified using octadecanol, and hydroxyalkanoic acids were quantified using 16-hydroxyhexadecanoic acid.

Based on the literature (Otto *et al.*, 2005; Pollard *et al.*, 2008) and the biomarkers extracted from the litter of the study species (Fig. S1), the compounds were grouped as short-chain fatty acids (ΣSFA; C_{10}–C_{18} *n*-alkanoic and *n*-alkanedioic acid), long-chain fatty acids from plant waxes (ΣLFA; > C_{22} alkanes, > C_{22} *n*-alkanoic acids and alkanols), cutin (ΣCutin; C_{14}–C_{18} hydroxyalkanoic acids, C_{16}- di-hydroxyalkanoic acids, C_{16}–C_{18} ω-hydroxy- and ω-hydroxy-epoxy alkanoic acids), suberin (ΣSuberin; α,ω-dicarboxylic acids in the range of C_{16}–C_{30} (saturated and substituted)), and ω-hydroxyalkanoic acids (C_{20}–C_{30}; saturated and substituted).

Hydrogen peroxide oxidation to test the stability of SOM

The stability of the SOM was assessed by oxidizing the nonresilient C using 10% hydrogen peroxide (H_2O_2), the validity of which has been previously confirmed by radiocarbon dating, infrared spectroscopy, and NMR spectroscopy studies (Eusterhues *et al.*, 2005; Helfrich *et al.*, 2007; Favilli *et al.*, 2008). Two grams of air-dried soil was incubated with 100 ml of 10% H_2O_2 for 10 d at 50°C with frequent shaking. Oxidized samples and the nonoxidized, control soils were air-dried, finely ground and analysed for stable C isotope ratios (^{13}C to ^{12}C), %C, and %N, using an elemental analysis continuous flow isotope ratio mass spectrometer. The soil C content was corrected for variation in bulk density. The results of the isotope analysis were expressed as $\delta^{13}C$, where

$$\delta^{13}C\,(‰) = \frac{C^{13}/C^{12}\,\text{sample} - C^{13}/C^{12}\,\text{standard}}{C^{13}/C^{12}\,\text{standard}} \times 10^3 \qquad \text{Eqn 1}$$

Statistical analyses

Within each site, a mixed-model restricted maximum likelihood analysis was used to compare the main and interactive effects of invasion (invaded (IN) and adjacent noninvaded (OUT)) and depth (0–5, 5–10, and 10–15 cm) on the concentration of SOM components (organic C, ergosterol, muramic acid, biomarkers, lignin monomers, proportion of oxidation-resistant C and N, and changes in $\delta^{13}C$ value and C to N ratios). Six invaded stands within a site that represented independent experimental units were included as a random factor for all analyses. Principal component analysis, heatmaps and two-way hierarchical clustering (Ward's minimum-variance for linkages and Pearson's dissimilarity as distance measure) were used for the comparison of variation in the abundance of biomarkers (C normalized, free and bound lipids and phenolics combined) between invaded and noninvaded soils within a site. Data were scaled and log-transformed as needed to correct for heteroscedasticity before the multivariate analyses (van den Berg *et al.*, 2006). Statistically significant differences ($\alpha < 0.05$) were further subjected to Tukey's HSD multicomparison test.

Results

Quality of plant inputs

Invaded soils and the adjacent noninvaded soils within knotweed and kudzu sites exhibited similar soil physical characteristics (Tables 1, 2), which could be attributed to the homogenization of the soil profile during extensive tillage practices undertaken during previous crop production. Annual above-ground litter input in knotweed-invaded soils was 2.5 times higher than in the adjacent noninvaded soils (Table 1). Compared with the noninvaded soils, the below-ground root production, though similar in 0–5 cm soils, was 50% and 25% lower in knotweed-invaded soils at 5–10 and 10–15 cm depths, respectively. Compared with the old-field species, knotweed litter was three times lower in N per unit of C, fourfold higher in lignins, and twofold higher in cutin, and contributed > 100 g tannins m^{-2} yr^{-1} (Table 1). In the *Pinus* forest, kudzu-invaded and adjacent noninvaded soils received similar amounts of above-ground *Pinus* litter (Table 2). In addition, the invaded soils received an additional 22% of kudzu litter that had seven times more N per unit of C, eight times lower lignin content and low polyphenol content compared with *Pinus* litter (Table 2). Thus, based on our study site selection, the knotweed-invaded system represented encroachment of an invader that input recalcitrant litter into an old-field ecosystem adapted to labile inputs, and kudzu invasion represented encroachment of an invader that input labile litter into an ecosystem that is adapted to recalcitrant inputs from *Pinus*.

Soil C accrual and microbial abundance in invaded ecosystems

In both the study systems, the invaded soils differed significantly from the noninvaded soils with respect to soil C content (corrected for variation in soil bulk density) and microbial biomass indicators (Fig. 2). In general, the differences between knotweed-invaded and adjacent noninvaded old-field soils were limited to the top 10 cm, whereas the kudzu-invaded soils

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Table 2 Characteristics of soils[1] and litter[2] input in the *Pueraria lobata* invaded site[3]

	0–5 cm		5–10 cm		10–15 cm	
	Invaded	Noninvaded	Invaded	Noninvaded	Invaded	Noninvaded
Soil pH	5.1 (0.03)[a]	5.0 (0.1)[a]	5.2 (0.07)[a]	5.1 (0.06)[a]	5.2 (0.03)[a]	5.1 (0.04)[a]
Sand (%)	61.9 (1.6)	60.5 (1.2)	65.5 (1.5)	66.1 (1.4)	59.3 (1.1)	58.4 (1.5)
Silt (%)	23.8 (0.5)	22.4 (0.8)	18.5 (1.1)	18.2 (1.0)	17.0 (0.5)	16.8 (1.3)
Clay (%)	14.3 (1.1)	14.8 (0.9)	16.1 (0.5)	15.9 (1.1)	23.7 (1.5)	22.9 (0.9)
Bulk density (Mg m^{-3})	1.23 (0.07)[a]	1.23 (0.04)[a]	1.23 (0.02)[a]	1.24 (0.07)[a]	1.29 (0.06)[a]	1.24 (0.08)[a]
Soil N (Mg ha^{-1})	0.44 (0.05)[a]	0.40 (0.06)[b]	0.30 (0.02)[a]	0.26 (0.01)[b]	0.22 (0.04)[a]	0.20 (0.02)[a]
Root biomass (g m^{-2})	281 (26)[a]	249 (28)[a]	293 (22)[a]	210 (16)[b]	235 (35)[a]	171 (36)[b]
Shoot biomass (g m^{-2})	Pinus- 94 (16)[a] Kudzu- 38 (8)	Pinus- 105 (14)[a]				
Biomass C : N	Pinus- 106 (11)[a] Kudzu- 16 (4)	Pinus- 118 (12)[a]				
Biomass lignin (%)	Pinus- 8.2 (1.1)[a] Kudzu- 1.0 (0.2)	Pinus- 8.1 (1.6)[a]				
Biomass tannin (%)	Pinus- 8.6 (0.9)[a]	Pinus- 8.1 (0.8)[a]				
Biomass lipids (%)	Pinus- 1.1 (0.5)[a] Kudzu- 0.3(0.06)	Pinus- 0.98 (0.2)[a]				

[1]Typic Kanhapludult, described as a pacolet fine sandy loam soil.
[2]Litter input was measured by collecting the freshly senesced above-ground litter within the sampling plots.
[3]Values represent mean and standard deviation. Parameters within the same depth were compared with one-way ANOVA; significant differences ($P < 0.05$) between values within a depth are represented by differing superscript letters.

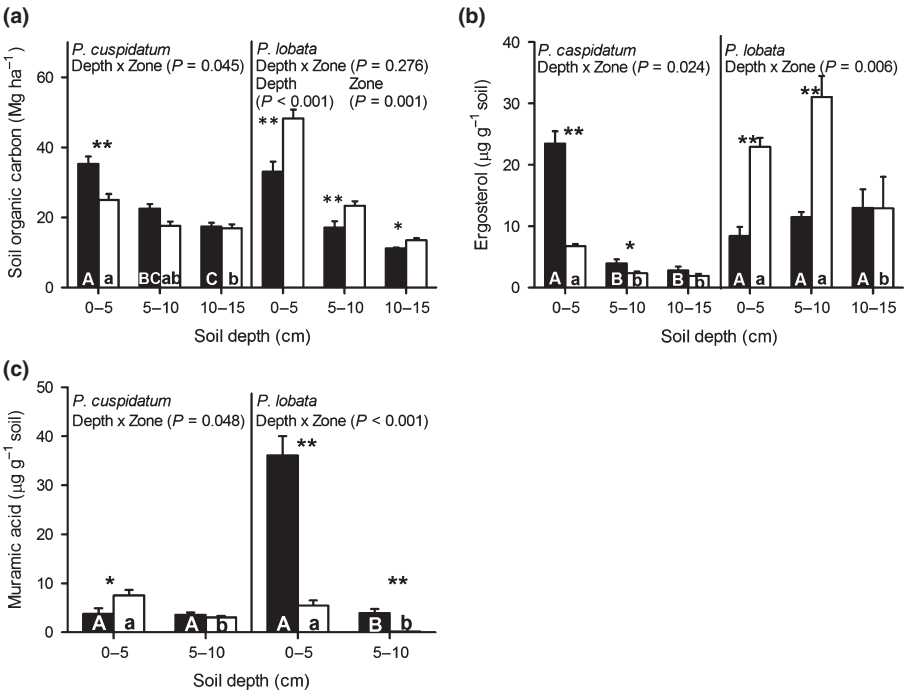


Fig. 2 Soil carbon and microbial biomarkers in *Polygonum cuspidatum* and *Pueraria lobata* invaded (IN; closed bars) and adjacent noninvaded (OUT; open bars) mineral soils. (a) Soil organic carbon content; (b) soil ergosterol (fungal biomass indicator); (c) muramic acid (bacterial biomass indicator) content (bars represent mean ± SE; $n = 6$). Within a zone (IN/OUT), bars with the same letters are not different ($\alpha = 0.05$). Within a depth, the symbols above pairs of bars shows whether means are different between zones (*, $P = 0.05$–0.01; **, $P < 0.01$).

differed from the adjacent noninvaded *Pinus* soils throughout the measured 0–15 cm depth (Fig. 2). Knotweed-invaded soils had a 40% ($P < 0.001$) and 26% ($P = 0.058$) higher C content at 0–5 and 5–10 cm depth, respectively, compared with noninvaded soils inhabited by grasses and forbs, resulting in an overall 26% increase in soil C in invaded soils (0–15 cm; $P = 0.01$). Even though the above-ground litter input in kudzu-invaded soils was 22% higher than in the adjacent noninvaded soils under *Pinus* (Table 2), the C content of the kudzu-invaded soils decreased by 31.6, 26.6 and 17.0% at 0–5 ($P < 0.001$), 5–10 ($P < 0.001$) and 10–15 ($P = 0.01$) cm depths, respectively, resulting in an overall 28% decrease (0–15 cm; $P = 0.004$) in soil C.

The fungal and bacterial abundances in both the study systems were inversely related to each other (Fig. 2b,c). Knotweed-invaded soils (0–5 cm) exhibited a three times higher ergosterol content and a 50% reduction in muramic acid compared with the adjacent noninvaded soils, whereas the kudzu-invaded soils

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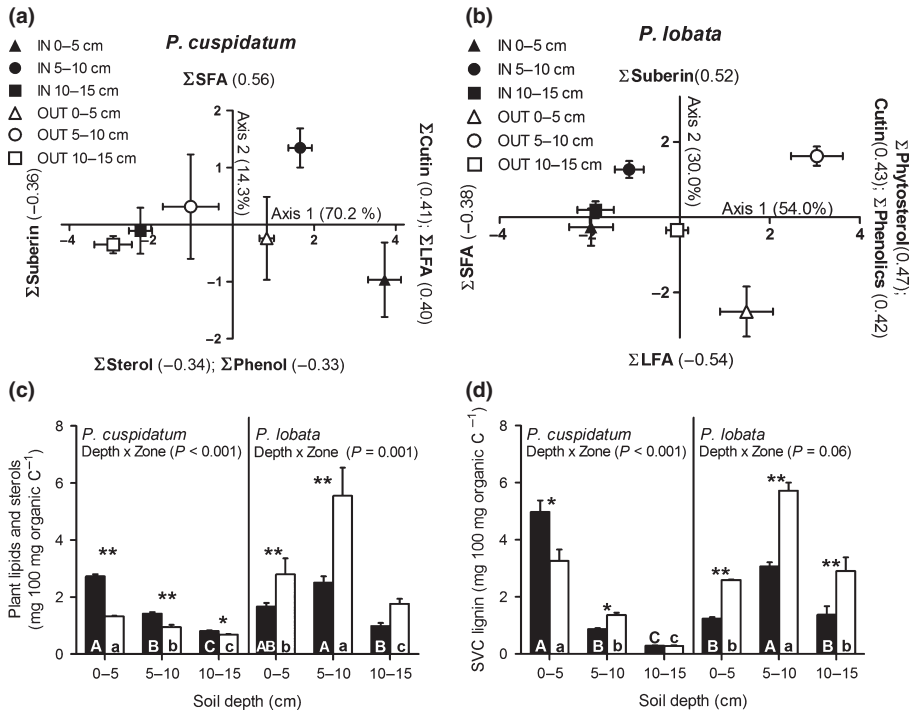


Fig. 3 The composition of the extractable soil organic matter (SOM) as assessed by the biomarker analyses in *Polygonum cuspidatum* and *Pueraria lobata* invaded (IN) and adjacent noninvaded (OUT) mineral soils. Principal component analysis of lipid and phenolic biomarkers under (a) knotweed invasion and (b) kudzu invasion (eigenvector loadings of the respective axis shown in parentheses; *post hoc* mean separation (Tukey's HSD) test in Supporting Information Table S2; $n = 3$–4); (c) abundance of total plant biomarkers (d) concentration of total lignin monomers after CuO oxidation (SVC represents the concentration of syringyl, vanillyl, and cinnamyl phenols). Closed bars, IN; open bars, OUT. Bars represent mean ± SE; $n = 6$. ΣSFA, short-chain fatty acids (C_{10}–C_{18} *n*-alkanoic and *n*-alkaenoic acid); ΣLFA, long-chain fatty acids (> C_{24} alkanes, > C_{22} *n*-alkanoic acids and alkanols); ΣCutin, (C_{14}–C_{18} hydroxyalkanoic acids, C_{16}- di-hydroxyalkanoic acids, ω-hydroxy- and ω-hydroxy-epoxy alkanoic acids (C_{16}–C_{18})); ΣSuberin, (α,ω-dicarboxilic acids (C_{16}–C_{24}; saturated and substituted) and ω-hydroxyalkanoic acids (C_{20}–C_{30}; saturated and substituted)). Within a zone (IN/OUT), bars with the same letters are not different (α = 0.05). Within a depth, the symbols above pairs of bars shows whether means are different between zones (*, $P = 0.05$–0.01; **, $P < 0.01$).

(0–10 cm) exhibited a 64% reduction in ergosterol content and a ninefold increase in muramic acid content compared with the adjacent noninvaded *Pinus* soils ($P < 0.01$).

Molecular composition of SOM as assessed by biomarker analysis

The concentrations of 82 compounds, including fatty acids, sterols, and phenolics, normalized by the soil C content, were used to assess the molecular composition of the extractable SOM fraction (Fig. 3a,b). The PC-1 axis which accounted for 70% of the variation separated the 0–5 cm depth of the knotweed-invaded soils (Tukey's HSD; $P < 0.05$; Tables S1–S4), which was abundant in lipids originating from cutin and plant waxes (Figs 3a, S2a). The compositional similarities of biomarkers between depths of 5–10 cm in invaded soils and 0–5 cm in noninvaded soils, and between depths of 10–15 cm in invaded soils and 5–10 and 10–15 cm in noninvaded soils (Fig. 3a, Table S2), indicated that the potential impact of knotweed invasion on the composition of extractable SOM is confined to the top soil layer. The lower depths of both knotweed-invaded and noninvaded soils were abundant in suberin-derived lipids (Figs 3a, S2a). Compared with noninvaded old-field soils, the knotweed-invaded soils exhibited 2- and 1.3-fold increases in total plant biomarkers (lipids derived from cutin, suberin, waxes and sterols) in the top 0–5 and 5–10 cm soil layers, respectively (Fig. 3c). Knotweed-invaded soils exhibited a 42% increase and a 25% decrease in lignin monomers at 0–5 and 5–10 cm, respectively (Fig. 3d), and a 4 times and 3.5 times lower degradation rate of plant sterols (assessed using the ratio of the concentration of the product (stigmasta-3,5-dien-7-one and sitosterone) to that of the precursor (beta-sitosterol and stigmasterol) sterol; Otto *et al.*, 2005) at 0–5 and 5–10 cm, respectively (Fig. S3).

By contrast, the variation in the chemical composition of extractable SOM across soil depths was observed in noninvaded kudzu soils under *Pinus* (Tukey's HSD; $P < 0.05$; Figs 3b, S2b, Table S2b). The PC-1 axis which explained 54% of the variation in the biomarker data set separated the noninvaded 0–10 cm soil layer from the rest, and these soils were abundant in phytosterols, cutin and phenolics (Fig. 3b; PC-1 axis; Tukey's HSD; $P < 0.05$; Table S2a). Kudzu-invaded soils, across all depths, were abundant in short-chain fatty acids that are characteristic of microbial biomass. The PC-2 axis which explained 30% of the variation separated the 0–5 and 5–10 cm depths of both invaded and noninvaded soils. The 0–5 cm depth of both invaded and noninvaded soils was abundant in lipids derived from plant waxes, whereas 5–10 cm soils were abundant in lipids derived from suberin (Figs 3b, S2b). Despite a similar input of *Pinus* litter in both

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kudzu-invaded and noninvaded soils (Table 2), the kudzu-invaded soils were lower in plant-derived lipids by 40.2% ($P = 0.007$), 54.9% ($P < 0.001$) and 44.4% ($P = 0.079$) at 0–5, 5–10 and 10–15 cm, respectively (Fig. 3c). Similarly, kudzu-invaded soils across all depths had a 50% lower concentration of lignin monomers ($P < 0.05$; Fig. 3d), and the highest reduction was observed for gymnosperm-derived vanillyls units (63%; $P = 0.003$; Fig. S4) of lignin.

Soil C stability as assessed by resistance to oxidative degradation and the associated shifts in C isotopic abundance

Oxidation with H_2O_2 delineated the differences in C stability between invaded and noninvaded soils, which varied with depth ($P < 0.05$). Compared with their respective noninvaded soils, the knotweed-invaded soils had a 21% lower proportion of stable C in the top 0–5 cm ($P < 0.001$; Fig. 4a), whereas kudzu-invaded soils had a 51% higher proportion of stable C ($P = 0.006$). The delta ^{13}C values of the leaf litters of all study species (knotweed, grass/forbs, kudzu, and *Pinus*) ranged from −25.5 to −28.5‰, which are typical for C_3 species (Fig. S5). Upon oxidation, the 0–5 cm depth of knotweed-invaded soils exhibited a 29% higher $\delta^{13}C$ enrichment compared with the adjacent noninvaded soils (Fig. 4b). Despite a similar amount of C to that in the noninvaded soils (Fig. 2a), the proportion of oxidation-resistant C was 27% and 33% higher in the 5–10 and 10–15 cm depths of knotweed-invaded soils, respectively ($P < 0.01$; Fig. 4a). Kudzu-invaded and noninvaded soils exhibited similar $\delta^{13}C$ enrichment upon H_2O_2 oxidation. Compared with their respective noninvaded soils, the knotweed-invaded soils had a lower proportion of oxidation-resistant N (27%; $P = 0.09$), whereas the kudzu-invaded soils had a 2.5-fold higher proportion of stable N (Fig. 4c). Overall, the C : N of the oxidation-resistant SOM was three times lower for kudzu-invaded soil (Fig. 4d).

Discussion

The litter quality of the invader influences soil C accrual and microbial abundance

The influence of invasion and litter quality on soil C accrual was evident, especially in the top layer of the soil, where soils that received input of recalcitrant litter (knotweed-invaded soils and noninvaded *Pinus* soils) accrued more C. This could partly be attributed to the abundance in the input of polyphenolic and cuticular materials to these soils (Tables 1, 2, Fig. S1). The heteropolymeric compounds that pose variable linkages between monomers are slower to decompose than homopolymeric compounds, such as cellulose, with homogeneous linkages (Feng *et al.*, 2008; Eastwood *et al.*, 2011). Thus, over time, recalcitrant compounds with inputs that steadily exceed their rate of mineralization might persist in the soil and contribute significantly to the SOM (Lorenz *et al.*, 2007; De Deyn *et al.*, 2008). A similar five-fold increase in soil C concentration in grasslands following prolonged encroachment of *Prosopis glandulosa* (Liao *et al.*, 2006) has been partly attributed to the preservation of recalcitrant compounds of the invader (Filley *et al.*, 2008). A gradient increase in soil C (0–5 cm) from the noninvaded old-field soils towards the centre of the established knotweed stands was also observed in other knotweed-invaded old-field sites in the eastern USA (Fig. S6), confirming comparable SOM dynamics in similar knotweed-invaded ecosystems. A similar gradient increase in the thickness of the litter layer has been reported from knotweed-invaded sites in Europe (Maurel *et al.*, 2010), indicating a slower turnover of knotweed litter in other ecosystems.

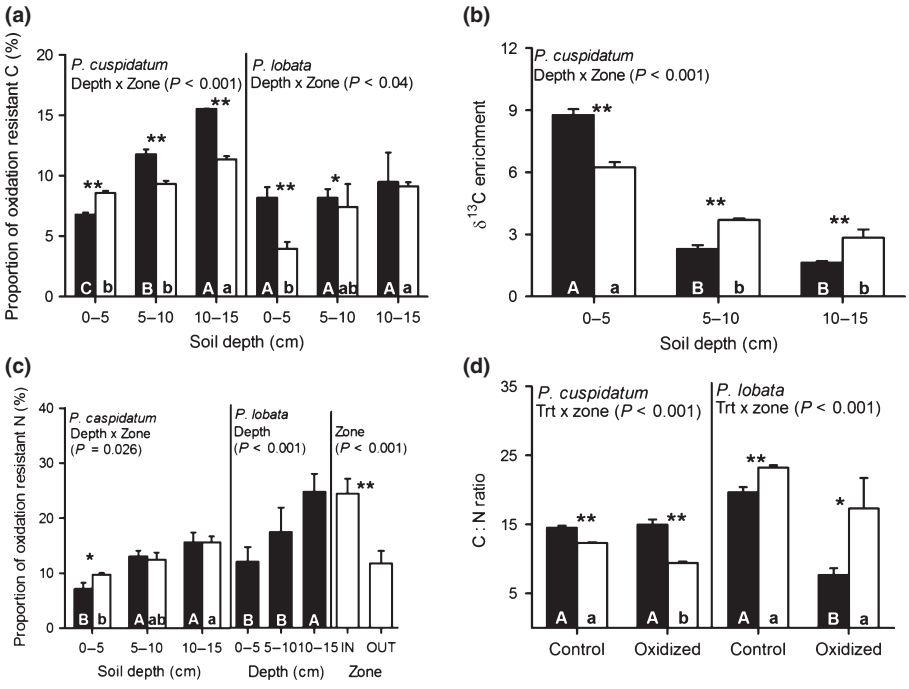


Fig. 4 The stability of soil organic matter (SOM) (assessed by 10% H_2O_2 oxidation) in *Polygonum cuspidatum* and *Pueraria lobata* invaded (IN; closed bars) and adjacent noninvaded (OUT; open bars) mineral soils (bars represent mean ± SE; $n = 6$). (a) Proportion of oxidation-resistant carbon (C); (b) enrichment of $\delta^{13}C$ values; (c) oxidation-resistant nitrogen (N); (d) C : N ratio of nonoxidized (control) and H_2O_2-oxidized soils (0–15 cm). Within a zone (IN/OUT), bars with the same letters are not different ($\alpha = 0.05$). Within a depth, the symbols above pairs of bars shows whether means are different between zones (*, $P = 0.05$–0.01; **, $P < 0.01$).

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Litter quality also influenced the microbial composition at both sites; the knotweed-invaded soils and the noninvaded kudzu soils that received recalcitrant litter exhibited a higher fungal abundance and lower bacterial abundance. Higher abundance of recalcitrant substrates promotes fungal communities as a result of a greater ability of fungi to mineralize plant heteropolymers (Sinsabaugh, 2010). The C-use efficiency of fungi is higher (Six *et al.*, 2006; Sinsabaugh *et al.*, 2013), especially when utilizing recalcitrant substrates (Frey *et al.*, 2013), and thus could facilitate the storage of C in soils (Clemmensen *et al.*, 2013) that receive heteropolymeric inputs. Further, as a result of accompanying lower cycling of mineral nutrients in these ecosystems (Hattenschwiler & Vitousek, 2000; Hattenschwiler *et al.*, 2011; Tharayil *et al.*, 2013), most of the resident plant species form abundant mycorrhizal associations for nutrient uptake, which also may contribute to the observed increase in soil C stocks (Langley & Hungate, 2003; Clemmensen *et al.*, 2013). Fast-growing bacterial communities can effectively outcompete fungal communities in the abundance of labile substrates (McGuire & Treseder, 2010), which is corroborated by a higher abundance of muramic acid in noninvaded knotweed soils and kudzu-invaded soils.

As kudzu was invading actively growing *Pinus taeda* stands, the kudzu-invaded soils received an overall 22% higher litter input than adjacent noninvaded soils under *Pinus*. Despite this additional litter input, the observed 28% decrease of C in invaded soils indicates a potential microbial priming effect leading to the loss of SOM under kudzu invasion. The invasion of kudzu, by providing more labile substrates that facilitate the production of energy-demanding oxidative enzymes, could initiate decomposition of *Pinus*-derived SOM that is otherwise less prone to degradation. The abundance of organic forms of N could stimulate the production of laccase, an oxidative enzyme that depolymerizes lignins (Piscitelli *et al.*, 2011), which could facilitate the observed priming of SOM under kudzu invasion. Such co-metabolism of recalcitrant compounds in the presence of labile substrates has been reported previously in soils (Phillips *et al.*, 2012) and during decomposition of mixed litters (Gartner & Cardon, 2004), leading to the loss of C from ecosystems (Fontaine *et al.*, 2007). Although the addition of inorganic N is traditionally thought to promote selective preservation of recalcitrant compounds in soil (Zak *et al.*, 2008), recent modelling studies indicate that elevated C stocks under N deposition might be attributable to accelerated but incomplete litter decomposition (Whittinghill *et al.*, 2012), which supports the possibility of microbial priming in kudzu-invaded soils. Similarly, compared with the deposition of inorganic N, the deposition of organic forms of N has been shown to accelerate soil C cycling and the loss of C from soils (Du *et al.*, 2014). The incorporation of N-rich leguminous litter is thought to increase the soil C sequestration potential of agro-ecosystems (Drinkwater *et al.*, 1998), partly as a result of the higher overall biomass production of the cropping system (Bayer *et al.*, 2009). The loss of C observed under the invasion of leguminous kudzu in our study system could partly be attributable to the lack of increase in litter input by *Pinus* in kudzu-invaded soils even in the presence of higher soil N in the top 0–10 cm (Table 2). The lower productivity of this invaded system despite the high availability of N could be attributed to the partial shading of *Pinus* by kudzu during the active growing season (June–October). A similar priming of native C from Ultisols upon mulching with legume litter was reported by Barthès *et al.* (2004) and Pascault *et al.* (2013).

Plant litter chemistry influences the soil organic matter composition

The distribution of plant and microbial biomarkers reflected the influence of the metabolic inputs of dominant plant species on the composition of SOM – that is, the soils receiving a high input of recalcitrant litter (knotweed-invaded soils and noninvaded *Pinus* soils) were characterized by the abundance of plant-derived polymers, indicating the persistence of recalcitrant biopolymers. Knotweed-invaded soils exhibited a higher persistence of cutin and waxes at 0–5 cm, whereas lipids derived from suberin dominated the 5–15 cm layer of the noninvaded soils and the 10–15 cm layer of the invaded soils, indicating higher root production at these depths. Similarly, the knotweed-invaded soils retained a substantial amount of un-decomposed form of plant sterols (Fig. S3), and this selective preservation of the cyclic structures of sterols is in agreement with Otto *et al.* (2005). The higher abundance of lignin at 5–10 cm in the noninvaded old-field soils could be attributed to the higher density of roots of grasses and forbs at this depth, which is supported by the pattern of suberin distribution. Combined with the observed increase in soil C (Fig. 2a), the biomarker analysis which verifies the abundance of plant lipids and polyphenols (Fig. 3a,c,d) supports the critical role of the selective preservation of plant polymers in the formation of SOM in knotweed-invaded soils. A similar, higher retainment of recalcitrant lipids of *Prosopis glandulosa* in invaded grasslands soils has been previously reported by Filley *et al.* (2008).

The recalcitrant nature of *Pinus* litter resulted in the formation of chemically distinct SOM at the three depths of noninvaded *Pinus* soils, where the compositional difference was contributed by a gradient in abundance of cutin-, wax- and suberin-derived lipids with depth. The higher content of plant biomarkers at 5–10 cm in noninvaded *Pinus* soils could be primarily attributed to higher suberin and lignin contents, which could result from a high root density at this depth. Despite a similar input of *Pinus* litter, the kudzu-invaded soils exhibited a loss of plant polymers and the homogenization of biomarkers across the three depths. Despite the 22% greater litter input into the kudzu-invaded soils, the abundance of plant biomarkers (lipids and lignin combined) decreased by 2.3-fold, and was accompanied by a 28% decrease in C in the 0–15 cm soil profile (Fig. 2a). Together, these observations indicate microbial priming of *Pinus*-derived SOM. The two times higher degradation of vanillyl units of lignin which are abundant in *Pinus* litter further supports the microbial priming of *Pinus*-derived SOM in kudzu-invaded soils (Fig. S4). Plant suberins and waxes persisted in the kudzu-invaded soils (Figs 3b, S2b), indicating the relative resistance of these polymers to microbial decomposition.

Overall, our results indicate that the amount of C accrued in invaded landscapes is tightly regulated by the amount and quality of the litter input by the dominant species, as well as the

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characteristics of native ecosystems; that is, the invasion of a recalcitrant species into an ecosystem adapted to relatively labile inputs could facilitate the storage of C in soils through selective preservation, whereas the invasion of a labile species into an ecosystem that is adapted to recalcitrant inputs could result in the loss of native soil C through microbial priming. Thus, even in ecosystems where the plant-derived compounds are less persistent in soils, the identity of dominant species nonetheless influences the SOM formation by potentially modulating the abundance and activity of heterotrophic soil biota. The impact of our studied species could vary based on the ecosystems they invade, with encroachment into native ecosystems of contrasting litter qualities resulting in maximal detrimental impacts.

The oxidation susceptibility of SOM is linked to the quality of the litter input

Compared with the soil C accrual, the stability of soil C exhibited an inverse relationship with the litter quality; that is, the soils that received input of recalcitrant litter (knotweed-invaded soils and noninvaded *Pinus* soils) had a lower proportion of stable C, whereas soils that received input of labile litter (noninvaded old-field soils and kudzu-invaded soils) exhibited a higher proportion of stable C. Thus, despite a 26% higher content of soil C, the amount of oxidation-resistant C in knotweed-invaded soils was similar to that of noninvaded old-field soils (Fig. S7). The $\delta^{13}C$ signature of SOM at 0–5 cm reflected the compositional difference in litter input at both invaded sites. The soils that received recalcitrant litter were relatively depleted of ^{13}C (Fig. S5), indicating a high abundance of lignin and lipids. As a result of isotopic fractionation during plant metabolism, the polymeric compounds, including lignin and long-chain lipids, are relatively depleted in ^{13}C compared with more labile compounds, such as cellulose (Balesdent *et al.*, 1987; Brüggemann *et al.*, 2011). The subsequent microbial decomposition of plant litter results in a further enrichment of ^{13}C in transformation products as a result of the preferential respiration of ^{12}C and microbial re-synthesis (Ngao & Cotrufo, 2011). Thus, combined with the biomarker analysis which revealed a high abundance of plant heteropolymers in the knotweed-invaded soils, the decrease in the proportion of oxidation-resistant C upon H_2O_2 treatment along with the concomitant $\delta^{13}C$ enrichment indicated a lower stability of knotweed-derived plant polymers. The higher proportion of oxidation-resistant C at 5–10 and 10–15 cm depths of knotweed-invaded soils may be partly a result of the leaching of N-rich dissolved organic compounds to lower depths (Tharayil *et al.*, 2013), which in turn could facilitate the microbe-mediated C stabilization processes (Kleber *et al.*, 2007; Cotrufo *et al.*, 2013). The lower $\delta^{13}C$ enrichment of the SOM at lower depths in knotweed-invaded soils upon oxidation (Fig. 4b) is consistent with the above role of microbial transformations in SOM stabilization.

A higher proportion of stable C in kudzu-invaded soils compared with the noninvaded *Pinus* soils could partly be attributable to the mineralization of easily degradable fractions of SOM during microbial priming (Fig. 4a). Along with the loss of plant polymers that is evident from the biomarker analysis, a higher enrichment of $\delta^{13}C$ in the nonoxidized kudzu-invaded soils (Fig. S5), despite a similar input of *Pinus* litter, further supports the loss of *Pinus*-derived C upon kudzu invasion through microbial priming. The kudzu-invaded soils exhibited a 35% higher content of oxidation-resistant C compared with the noninvaded soils ($P = 0.018$; Fig. S7), indicating that kudzu invasion facilitated the formation of stable SOM.

Compared with leaf litter, root litter is thought to be more recalcitrant to microbial decomposition (Freschet *et al.*, 2012). Thus, a potentially higher turnover rate of roots in kudzu-invaded plots could have influenced the contrast in litter quality between the invader and the resident species at lower soil depths. The kudzu-invaded and adjacent noninvaded soils did not exhibit any difference in $\delta^{13}C$ enrichment after H_2O_2 oxidation, possibly as a consequence of a higher persistence of suberin in kudzu-invaded soils as indicated by the biomarker analysis (Fig. 3b). Along with a higher recalcitrance of suberin and waxes (Lorenz *et al.*, 2007), the higher abundance of clay particles at the kudzu site might have provided greater physical protection to suberin (Heim & Schmidt, 2007) against microbial priming. In general, comparing across invaded and noninvaded soils at each site, our study highlights the coexistence of both selective preservation and humification under the same edaphic conditions, with the dominant plant species potentially regulating the amount and stability of the accrued C.

The relationship between the input and stabilization of N in soil was opposite to that of C; that is, the input of litter with lower N (knotweed and *Pinus* litter) resulted in a lower proportion of oxidation-resistant soil N, whereas the input of N-rich kudzu litter resulted in the stabilization of a higher proportion of the total N in soil (Fig. 4c). A three times lower C : N of the oxidation-resistant SOM in kudzu-invaded soils indicated a higher abundance of microbial transformation products, which was confirmed indirectly by the relative depletion of extractable plant-derived compounds in the biomarker analysis. The stabilization of N was also related to soil mineralogy, and a higher proportion of clay content at 10–15 cm soil depth (Table 2) in kudzu-invaded soils resulted in 30% higher stable N. Nitrogenous compounds in the soil may form strong, inner-layer associations with mineral surfaces, which would further promote the sorption of C compounds to form the outer layer of the organo-mineral associations (onion-layering model; Kleber *et al.*, 2007). Thus, the stabilization of C in soil might be promoted by the association of the transformed nitrogenous compounds with mineral surfaces, which would partly explain the higher proportion of oxidation-resistant SOM in kudzu-invaded soils.

While the distribution of plant species across landscapes is tightly regulated by edaphic and climatic factors, within sites with similar soil and climatic conditions, our study captures the contrasting influence of dominant plant species on the accrual, composition and stability of SOM. Our study shows that SOM is an ecosystem property that is intricately interconnected through the interactions between edaphic factors, autotrophic plants and soil heterotrophs, and within the biota, the dominant plant species could have a significant influence on SOM sequestration

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potentially by influencing the metabolism of soil heterotrophs. Our study reports several novel findings that have implications beyond invasion ecology. First, our study highlights the concurrent influence of dominant plant species on microbial priming, humification and selective preservation, with litter chemistry and the associated microbial metabolism concomitantly shaping the quantity, composition, and stability of the SOM. Secondly, extending the current notion that microbial priming of SOM could lead to soil C losses, our study demonstrates a higher stability of the humified residual SOM against oxidative degradation, potentially by facilitating a stronger mineral association, and provides empirical evidence that supports the Microbial Efficiency-Matrix Stabilization framework (Cotrufo *et al.*, 2013). Our results thus emphasize the need for land-use and vegetation management practices that augment the stability of SOM by promoting the microbe-mediated transformation of the biomass produced. Thirdly, our study highlights the understudied but damaging impact of invasive plants on ecosystems, specifically, their potential to feed back to climate change. Based on calculations on the spread of kudzu in the USA (3 Mha; Lindgren *et al.*, 2013) and the C loss from invaded soils observed in the present study, we estimate that kudzu invasion results in a loss of 4.8×10^6 Mg C yr^{-1}, which equates to the C sequestered annually by 5.9 Mha of US forests (see Method S2 for calculations). Thus, annually, each hectare of kudzu invasion could release C sequestered by 2 ha of US forests, and knotweed invasion results in the accrual of soil C that is more prone to oxidation. Considering that future warmer climates could result in rapid northward range expansion of both the species studied herein, with kudzu spreading to southern New England (Bradley *et al.*, 2010) and knotweed expanding into northern Canada (Bourchier & Van Hezewijk, 2010), our results suggest the potential for feedback of these invasive species to warmer climates.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Heatmaps and two-way hierarchical clustering of biomarker data from plant litter.

Fig. S2 Heatmaps and two-way hierarchical clustering of biomarker data from soils.

Fig. S3 Extent of degradation of plant sterols in invaded and noninvaded soils.

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Fig. S4 Acid to aldehyde ratio of the vanillyl units of lignin in invaded and noninvaded soils.

Fig. S5 $\delta^{13}C$ values of organic matter in invaded and noninvaded soils.

Fig. S6 Effect of distance from the edge of stand of knotweed on soil C at three old-field sites.

Fig S7 Organic carbon content in invaded and adjacent noninvaded soils after H_2O_2 oxidation.

Table S1 Results of ANOVA of principal component analysis of soil biomarker analysis

Table S2 Results of *post hoc* mean separation test from ANOVA of principal component axis

Table S3 Aliphatic lipids and phenols identified in solvent extracts of soil and plant litter

Table S4 Aliphatic lipids and phenols identified from the base hydrolysates of soil and plant litter

Methods S1 Detailed methods for chemical analyses.

Methods S2 Calculation of soil carbon budget under kudzu invasion.

Notes S1 Description of study species.

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