



Warming and Labile Substrate Addition Alter Enzyme Activities and Composition of Soil Organic Carbon

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Warming can increase the efflux of carbon dioxide (CO₂) from soils and can potentially feedback to climate change. In addition to warming, the input of labile carbon can enhance the microbial activity by stimulating the co-metabolism of recalcitrant soil organic matter (SOM). This is particularly true with SOM under invaded ecosystems where elevated CO₂ and warming may increase the biomass of invasive species resulting in higher addition of labile substrates. We hypothesized that the input of labile carbon would instigate a greater soil organic carbon (SOC) loss with warming compared to the ambient temperature. We investigated this by incubating soils collected from a native pine (*Pinus taeda*) forest to which labile carbon from the invasive species kudzu (*Pueraria lobata*) was added. We evaluated the microbial extracellular enzyme activity, molecular composition of SOC and the temperature sensitivity of soil CO₂ efflux under warming and labile carbon addition. After 14 months of soil incubation, the addition of labile C through kudzu extract increased the activity of β-1,4-glucosidase compared with the control. However, the activity of N-acetyl-β-D-glucosaminidase and fungal biomass (ergosterol) decreased with labile carbon addition. The activity of peroxidase increased with warming after 14 months of soil incubation. Although the carbon content of incubated soils did not vary with substrate and temperature treatments, the molecular composition of SOC indicated a general decrease in biopolymers such as cutin, suberin, long-chain fatty acids, and phytosterol with warming and an increasing trend of microbial-derived compounds with labile substrate addition. In soils that received an addition of labile C, the macro-aggregate stability was higher while the temperature sensitivity of soil C efflux was lower compared with the control. The increase in aggregate stability could enhance the physical protection of SOC from microbial decomposition potentially contributing to the observed pattern of temperature sensitivity. Our results suggest that warming could preferentially accelerate the decomposition of recalcitrant compounds while the addition of labile substrates could enhance microbial-derived compounds that are relatively resistant to further decomposition. Our study further emphasizes that global change factors such as plant invasion and climate change can differentially alter soil microbial activity and the composition of SOC.

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INTRODUCTION

Warming can accelerate the efflux of carbon dioxide (CO₂) from the soil carbon pool to the atmospheric pool, potentially creating a positive feedback to climate change (Field et al., 2007; Wu et al., 2011). The magnitude of this feedback is partly determined by the temperature sensitivity of the microbial decomposition of soil organic matter (Conant et al., 2008; Suseela et al., 2012). Microbial metabolism is also positively influenced by the addition of labile carbon from plant litter. The input of labile carbon could subsidize the energy requirement of microbes for the production of oxidative enzymes. This in turn could stimulate the co-metabolism of relatively recalcitrant SOM (Kuzyakov et al., 2000) leading to the loss of soil C. Thus, both warming and the input of labile substrates could accelerate soil CO₂ efflux, which could potentially feedback to climate change. Although the influence of warming and labile substrate addition on the quantity of soil carbon has been studied independently, less is known about the interactive effects of these two factors (Zhang et al., 2013) on soil carbon stocks and particularly the molecular composition of resulting SOC, which could have implications on the stability of soil carbon.

Global changes such as elevated CO₂ and climate warming could differentially influence the primary producers and microbial decomposers that in turn could affect SOC storage. Elevated CO₂ and warming often stimulates plant growth (Rustad et al., 2001; Jackson et al., 2009; Morgan et al., 2011) leading to a higher carbon input while warming directly influences microbial metabolism and extracellular enzyme activity through changes in soil temperature and/or moisture (Birgander et al., 2013). The interactive effects of these global change factors are particularly important for soil carbon pools in native ecosystems that have been subjected to the encroachment of exotic invasive plant species. This is because some of the invasive plant species possess greater physiological adaptability to warmer climates, enabling them to input a higher amount of labile biomass to the invaded ecosystems than the natives they displace (Barney et al., 2006; Liao et al., 2008; Laungani and Knops, 2009; Holcombe et al., 2010). Also, most of the invasive plant species exhibit a higher resource acquisition and resource use efficiency than the native species (Funk and Vitousek, 2007) producing a higher biomass per unit of available resources (Laungani and Knops, 2009). Moreover, the spread of invasive species is projected to increase exponentially with warmer climates due to climate-matching and superior physiology (Barney et al., 2006; Holcombe et al., 2010; Murray et al., 2012; Sorte et al., 2013). The longer growing season and better climate matching due to warming could result in increased input of fresh biomass that in turn facilitate higher microbial metabolism (Rothstein et al., 2004; Allison et al., 2010) reducing soil carbon storage in the invaded soils. Thus, the warming-induced increase in microbial metabolism can interact additively with the input of labile carbon by exotic plant species resulting in a greater impact on the storage of SOC in invaded ecosystems. The invasion of exotic plant species is a major global change factor that threatens the sustainability of native ecosystems. Previous studies have reported a decline in SOC in invaded ecosystems due to the input of labile carbon

(Strickland et al., 2010; Tamura and Tharayil, 2014). However, the magnitude of this loss of soil C and subsequent composition of SOC as influenced by warmer temperature and labile C addition remains less known, as are the molecular-level changes in soil C composition due to microbial co-metabolism of native SOM.

Soil organic matter is a complex mixture of substrates with varying quality and decomposability. The chemical composition of substrates strongly influences the microbial C use efficiency (CUE), which is the ratio of mineralized vs. incorporated C as microbial biomass or by-product (Kallenbach et al., 2019). Substrates with lower quality (high C:N) and higher structural complexity have lower microbial carbon use efficiency (Cotrufo et al., 2013) leading to greater loss of carbon. The decomposition of these lower quality substrates (recalcitrant substrates) is more sensitive to temperature as they have higher activation energy (Davidson and Janssens, 2006). Thus, warming could accelerate the CO₂ efflux from SOM by increasing the rate of enzymatic reaction and by reducing the microbial CUE (Conant et al., 2011). Considering the potential additive influence of the input of labile C and warmer temperatures on microbial metabolism, we hypothesized that the input of labile carbon would instigate a greater loss of soil carbon under warming than at ambient temperatures. Labile carbon addition would also alter the temperature sensitivity of soil carbon efflux. In the absence of labile carbon, a lower temperature sensitivity of microbially mediated soil carbon efflux has been reported (Yuste et al., 2003; Arevalo et al., 2010). However, other studies have reported that even under warming, the higher abundance of labile substrates in soil (Feng et al., 2008; Pisani et al., 2014) and plant litter (Suseela et al., 2013) reduced the decomposition of the recalcitrant fraction. In other words, the abundance of labile C may reduce the temperature sensitivity of SOM decomposition (Ghee et al., 2013). Moreover, the input of labile carbon and a higher rate of microbial by-product accumulation that enhances the aggregate stability may in turn reduce the temperature sensitivity of SOM degradation by reducing the accessibility of microbes to the substrate (Sollins et al., 1996; Park et al., 2007; Qin et al., 2019). Here we investigated the effect of warming and labile carbon addition on soil C efflux, the temperature sensitivity and the molecular composition of SOC. We also evaluated the combined effect of warming and addition of labile C substrates on microbial biomass and microbial extracellular enzyme activity. In this study, the difference in C quality between the labile substrate and the soil was achieved by adding labile litter-extracts (C:N = 19) from kudzu, a nitrogen-fixing invasive species (*Pueraria lobata*) to pine (*Pinus taeda*; C:N = 128) forest soil that was relatively enriched in recalcitrant compounds (Tamura et al., 2017).

MATERIALS AND METHODS

Collection and Preparation of Soil Samples

We collected the soil samples for the incubation study from a *Pinus taeda* stand at the Clemson Experimental Forest, Clemson, SC, United States (34°44'N, 82°50'W). The top 5 cm of the mineral soil was collected using a soil corer (10 cm in diameter)

after carefully removing the organic layer. Soils were then passed through a 2 mm-mesh sieve. A specimen cup (120 ml) was filled with 75 g of this soil and placed in a 1 L mason jar. At both temperature treatments (20 and 30°C), a set of 10 replicates received a substrate treatment of either water or kudzu extract every 2 months (see below for extract preparation) resulting in four treatments. We destructively harvested four and six replicates of each treatment at 6 and 14 months after incubation, respectively. The water content of the soil was maintained at 65% water holding capacity throughout the experiment by periodically weighing and adding nanopure water. The mason jars with the soil samples were periodically ventilated to maintain the CO₂ concentration to <5% in the headspace.

Labile Substrate (Kudzu Extract) Preparation

A labile substrate was prepared from fresh kudzu leaves collected from the invaded site in Seneca, SC (SC34°41'N, 82°53'W). Briefly, 20 g of fresh kudzu leaves were blended with 250 ml of double deionized water and the slurry was sequentially filtered through a glass filter (1.6 μm). To adjust the concentration of the leaf extract to be added to soils in each specimen cup, we added a known volume of the extract to a tin capsule, completely dried, and examined the C and N content using a continuous-flow isotope ratio mass spectrometer. The application of 2 ml of this extract provided 130 μg C g⁻¹ soil that resulted in the maximum microbial activity before saturation (Bastida et al., 2013; Ghee et al., 2013). The amount was also equivalent to ~0.25% of the total organic carbon in each specimen cup. The extract also provided 6.8 μg N g⁻¹ at each application. The extract was added every 2 months for the entire 14-month incubation period. To compare the change in the quantity of native soil C and N in the samples after six and 14 months of incubation, the amount of C and N supplied through applications of kudzu extract was subtracted from the samples destructively harvested at six and 14 months of incubation.

Soil Enzyme Assay

The enzyme assays were conducted 14 days after substrate application at six and 14 months after incubation to capture the response of microbial activity to temperature and substrate addition treatments. The activity of hydrolase enzymes such as β-1,4-glucosidase (BG) that degrades cellulose and N-acetyl-β-D-glucosaminidase (NAG) that degrades chitin and peptidoglycan (Sinsabaugh et al., 1992) were quantified as per Sinsabaugh et al. (1999). The peroxidase enzyme was quantified to determine the lignolytic activity in the soil (Kirk and Farrell, 1987). Briefly, 800 mg of soil was combined with 150 ml of 50 mM sodium acetate (pH 5.5) using a high-speed blender for 1 min. Two hundred microliter of the soil slurry was transferred into a black 96-well plate. The plate also included 250 μl of the buffer as a blank sample. The hydrolase enzymes (BG and NAG) were quantified by adding 50 μl of 4-methylumbelliferyl β-D-glucopyranoside and 4-methylumbelliferyl N-acetyl β-D-glucosaminide as substrates, respectively. We used 200 μl of buffer and 50 μl of 4-methyl umbelliferone as a reference

standard (Suseela et al., 2014). The enzyme activity was calculated following DeForest (2009). To quantify the activity of peroxidase, the above slurry containing buffer and soil (0.75 ml) was combined with 0.75 ml of the substrate L-3,4-dihydroxyphenylalanine (L-DOPA) and 0.2 ml of 0.3% hydrogen peroxide in a micro-centrifuge tube. For this assay, we used substrate with buffer (0.75 ml each) and slurry with buffer (0.75 ml each) as controls and a blank that contained 1.5 ml of the buffer alone. Both hydrolases and oxidases enzyme assay samples were incubated at 20°C for 2 h. At the end of 2 h of incubation, the hydrolase activity was quantified by measuring the absorbance at 365–450 nm (ex-em) using a microplate fluorimeter. The peroxidase activity was measured as absorption at 460 nm (ex) using a UV-VIS spectrophotometer.

Microbial Biomass Estimation

We estimated bacterial biomass in the soils harvested at six months by quantifying the muramic acid. Fungal biomass was estimated by the abundance of ergosterol (Montgomery et al., 2000) at six and 14 months after incubation. To quantify the ergosterol content in soils, briefly, 5 g of soil was refluxed with 7.5 ml of 0.14 M methanoic potassium hydroxide at 82°C in glass tubes for 40 min. The supernatant (5 ml) was acidified to pH < 2 with 0.75 M HCl. The tubes were centrifuged at 2,500 × g for 15 min to sediment the humic precipitates and 6 ml of the supernatant was extracted with 1.2 ml of hexane on a rotary shaker for 5 min. One milliliter of the hexane phase was collected into a glass vial and dried completely under nitrogen gas. The dried content was further reconstituted in 100 μL of 0.1 M potassium hydroxide in isopropyl alcohol. The concentration of the extract was determined using high pressure liquid chromatography (HPLC). With an Onyx C18 column (monolithic silica, 130 Å; 100 mm × 4.6 mm I.D.; Phenomenex, Torrance, CA, United States) and a methanol-water (99:1, v/v) solvent at a flow rate of 1 ml min⁻¹, the ergosterol was detected by the absorbance of conjugated double bond at 282 nm.

To extract muramic acid, briefly, 3 g of soil was hydrolyzed with 10 ml of 6 N HCl for 6 h at 100°C and were then acidified and centrifuged to remove the humic precipitates. Prior to analysis on HPLC, the samples were derivatized. We combined 400 μL of the extract with 50 μL of ortho-phthalaldehyde (OPA) reagent (1 M sodium borate containing 40 mM OPA and 0.01% of β-mercaptoethanol) and the reaction was ceased at 1 min using 30 μL of 10% acetic acid (Tharayil et al., 2013). The derivatized samples were immediately analyzed on HPLC using a Synergy Polar-RP column (ether-linked phenyl, 4 μm, 80 Å; 250 mm × 4.6 mm I.D.; Phenomenex, Torrance, CA, United States). We used a binary gradient elution of 5 mM ammonium acetate (mobile phase A) with a linear increase of mobile-phase B (100% methanol) from 0% at 0 min to 40% at 20 min at a flow rate of 0.5 ml min⁻¹. Muramic acid was quantified using a fluorescence detector (Ex-Em = 340 nm and 455 nm, respectively). Using a triple quadrupole mass spectrometer (reaction monitoring m/z 252- > 144 and m/z 252- > 126), the muramic acid in samples was verified.

Sequential Extraction of Lipid and Phenolic Biomarkers

Lipids and phenolics were extracted using solvent extraction, base hydrolysis, and copper oxide oxidation of the mineral soils to compare the composition of soil organic C sources as either plant or microbial origin (Otto et al., 2005). The biomarker approach can provide compound-specific information and facilitate the classification of compounds of the extractable fraction in SOM to plant/microbial origins (Simpson et al., 2008; Zhang and Suseela, 2021). Kudzu litter extract did not contain any measurable amount of recalcitrant plant polymers, thus no adjustment was made for the addition of kudzu extract on the concentrations of the lipids and phenolics.

Solvent Extraction of Free Lipids

We combined 300 mg of dry soil sample with 3 mL of 100% methanol and sonicated in water bath for 30 min. The sample was centrifuged, and the supernatant was collected. The residue soil sample was sequentially extracted with 3 mL of dichloromethane/methanol (1:1 v/v) and 100% dichloromethane by following the same procedure, and all the solvent extracts were combined. We added 7 ml of DDI water to the extract and the content was gently shaken. The methylene chloride phase (1.5 ml) was collected and completely dried. The samples were stored at -20°C until analysis.

Base Hydrolysis of Ester-Bound Lipids and Phenolics

To the subsamples of solvent extracted soil, 3 ml of 1N methanolic sodium hydroxide was added and heated at 95°C for 3 h. After cooling to room temperature, the supernatant was collected. The residue soil was combined with 2 ml of 100% methanol and sonicated in a water bath for 30 min, and the supernatant was combined with the previous base solvent. The extraction was repeated with 2 mL of methylene chloride. To the combined extract sample, 100 μl of nonadecane (100 $\mu\text{g ml}^{-1}$ in 100% methanol) was added as an internal standard. We added 2 ml of 50% HCl to the sample before adding 7 mL of DDI water. We collected 500 μl of the methylene chloride fraction and stored at -20°C until analysis.

CuO Oxidation of Lignin Monomers

Briefly, the base extracted soil equivalent to roughly 25 mg of soil C, was combined with 1 g CuO, 150 mg ferrous ammonium sulfate, and 15 ml of 2 M NaOH that had been sparged for 20 min with argon, into a 23 ml Teflon-lined acid digestion vessel. Further, each vessel was sparged for an additional 15 min before closing. The samples were heated to 160°C for 160 min and then allowed to cool to room temperature. Upon cooling, the supernatants were transferred and 100 μl of the internal standards (cinnamic acid and ethylvanillin prepared as 200 $\mu\text{g ml}^{-1}$ in 100% methanol) were added to each sample, followed by the addition of 3 ml of 18 N H_2SO_4 . Twelve milliliters of the supernatant was extracted with 1 ml of ethyl acetate, and the ethyl acetate fraction was collected and stored at -20°C for further analysis.

GC-MS Conditions for Biomarker Analysis

The dried extract from the solvent extraction was reconstituted in 1 ml of methanol and methylene chloride (1:1) solution. Fifty microliters each of free and bound lipid and lignin extract were each transferred separately to glass vials with 250 μl inserts and completely dried under nitrogen gas. The samples were then silylated with 200 μl of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) with 1% trimethylchlorosilane (TMCS) at 65°C for 30 min. The silylated samples were analyzed using an Agilent 7980A GC system coupled with a 5975 C Series mass detector. The separation of the compounds was achieved on a DB-5 MS fused-silica capillary column (30 m length \times 0.25 mm internal diameter \times 0.20 μm film thickness) using a split (1:10) injection (1 μl). For the free and bound lipids, the samples were run at 65°C for 2 min followed by a linear ramp of 6°C per min to 300°C and held at 300°C for 15 min. The samples for lignin estimation were analyzed at 80°C for 2 min followed by a linear ramp of 10°C per min to 300°C . The carrier gas (He) was maintained at a constant pressure of 10 psi, the injection port and the MS interphase were maintained at 280°C , the MS quad temperature was maintained at 150°C and MS source temperature was set at 230°C . The electron multiplier was operated at a constant gain of 10 (EMV = 1478V) and the scan range was set at 50–600 amu resulting in 2.66 scans sec^{-1} .

Macro-Aggregate Stability

Aggregate stability was assessed by chemical dispersion as per Wright and Upadhyaya (1998). Briefly, the soil was wet sieved using a 250 μm sieve to obtain the macro-aggregate fraction (250–2,000 μm). Four grams of the macro-aggregate fraction was then mixed with 50 ml of 5% sodium hexametaphosphate, and dispersed on a rotary shaker for 20 h at 20°C . The samples were centrifuged, and supernatant was discarded. The residue soil fraction was dried at 45°C and weighed. The initial and final weight of the macroaggregate sample was used to calculate the stable aggregate fraction. The aggregate stability was conducted only on soils harvested at 6 months since soils incubated for 14 months were prioritized for all other analyses.

Carbon Dioxide Efflux Quantification

We measured CO_2 efflux from soils incubated for 12 months immediately after the application of substrate (kudzu extract or water treatments). Initially, the measurements were taken at times 0, 9, 24, 33, and 48 h with subsequent measurements taken every 48 h and terminated at 1,008 h (42 days). To measure the CO_2 concentration, 5 ml of the head space of the mason jar was collected using a gas tight syringe and injected into an infrared gas analyzer (EGM-4, PP-Systems, Amesbury, MA, United States). The data was recorded as an average of two consecutive measurements for each sample. Temperature sensitivity of the water and kudzu applied soils expressed as Q_{10} was calculated using the following equation:

$$Q_{10} = \left(\frac{C_2}{C_1} \right)^{10/T_2 - T_1} \quad (1)$$

where T1 is the base temperature (20°C) and T2 is the elevated temperature (30°C). C1 and C2 are the CO₂ concentrations of 20 and 30°C soil within the same substrate treatment, respectively.

Statistical Analysis

The data were log transformed wherever necessary to meet the assumptions of normality. Two-way analysis of variance (ANOVA) was conducted to test the main and interactive effects of temperature (20 or 30°C) and substrate (water or kudzu extract) treatments on soil organic C, carbon to nitrogen ratio, enzyme activity, microbial biomass, plant and microbial biomarkers, and aggregate stability from the samples destructively harvested at six and 14 months of incubation. Statistically significant differences ($\alpha < 0.05$) were further subjected to Tukey’s HSD multi-comparison test to obtain difference between individual treatments.

RESULTS

Quantity of Soil Carbon

At six months, both temperature and substrate treatments did not affect the total carbon content of the incubated soils (Supplementary Figure 1). However, at 14 months, the carbon content of pine soil exhibited a decreasing trend in soils incubated at 30°C compared with soils at 20°C but was not significant ($P = 0.13$; Supplementary Figure 1).

Microbial Biomass

Indicators of fungal and bacterial biomass (ergosterol and muramic acid, respectively) were quantified from the soil samples destructively harvested at six months after incubation. The concentration of ergosterol varied with the main effect of temperature (Figure 1A) and substrate treatments (Figure 1B). The ergosterol content of soils incubated at 30°C was 50% less than soils incubated at 20°C (Figure 1A; $P = 0.02$) and

soils amended with kudzu extract had higher ergosterol content than soils added with water (control; Figure 1B; $P = 0.05$). The concentration of muramic acid showed a decreasing trend with the application of kudzu extract (Figure 1C; $P = 0.07$). At 14 months after incubation, higher amount of ergosterol was quantified from water applied soil (Figure 1D; 35%) than the kudzu applied soil ($P = 0.03$).

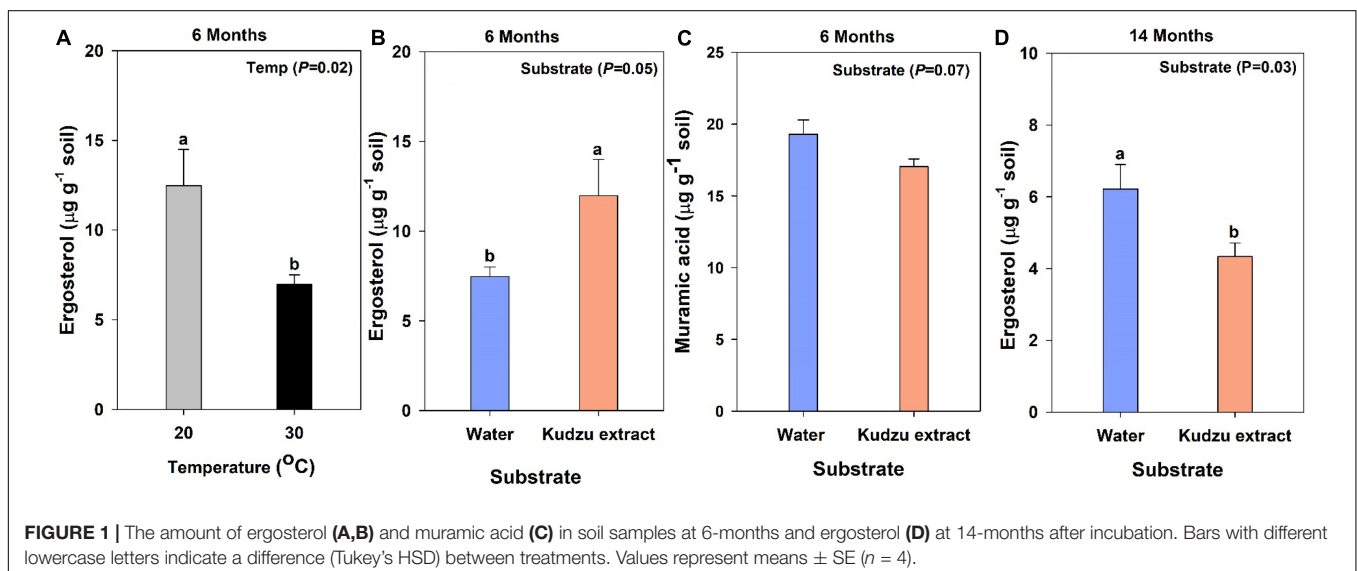
Soil Enzyme Activity

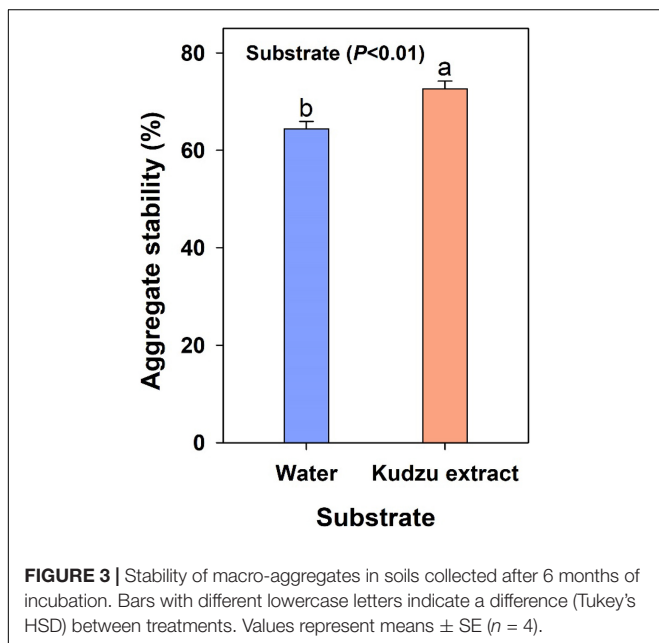
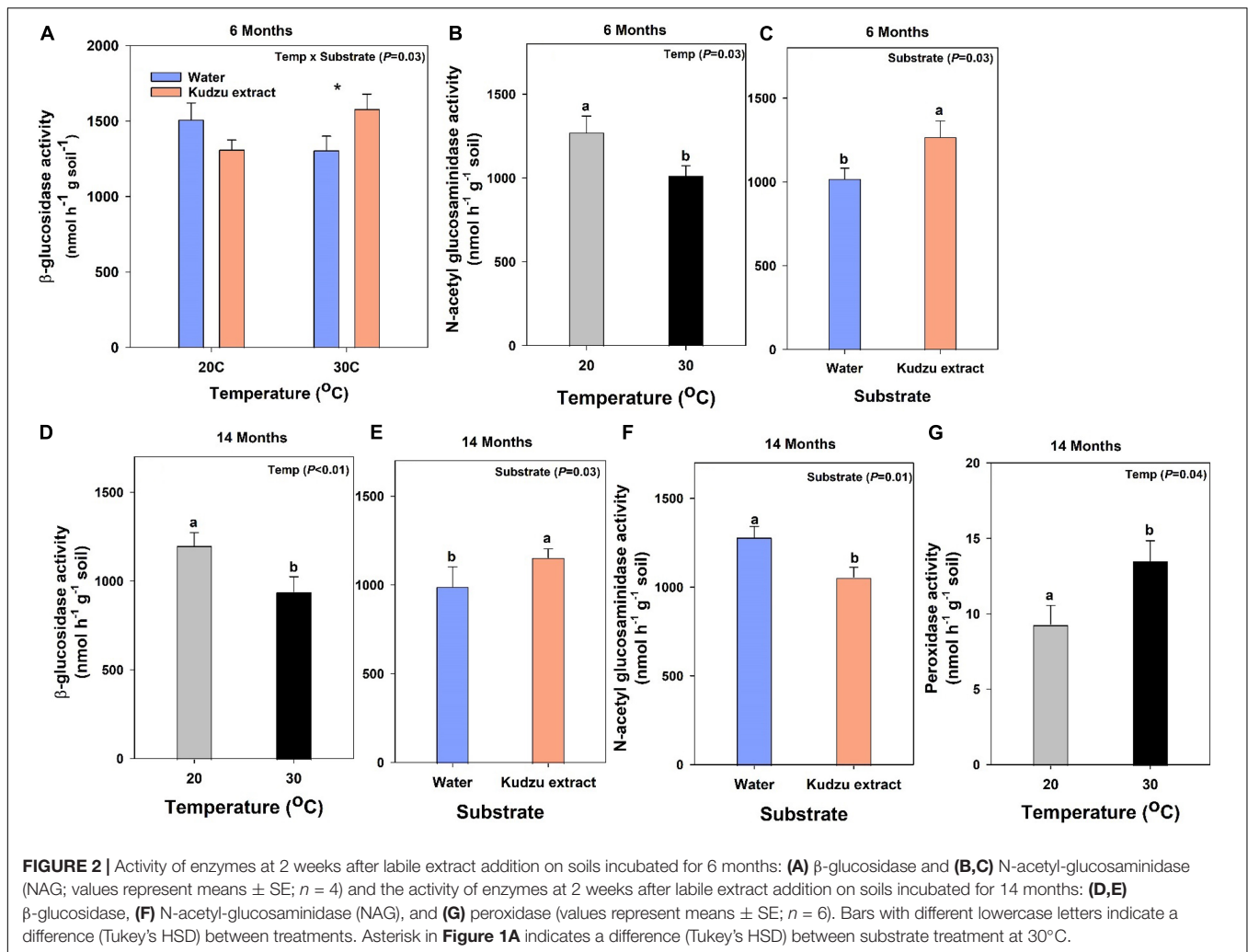
At six months after incubation, β -glucosidase, a cellulolytic enzyme, exhibited higher activity (23%) in soils that received kudzu extract and incubated at 30°C (Figure 2A; $P = 0.03$). The activity of NAG, a chitinase enzyme had 27% higher activity than soils at 30°C ($P = 0.03$) and soils that received the input of kudzu extract had 25% higher activity than the control ($P = 0.03$, Figures 2B,C). However, the activity of peroxidase, one of the lignolytic oxidative enzymes did not vary with treatments ($P > 0.05$; data not shown) after six months of incubation.

The enzyme activities in soils harvested at 14 months did not follow the trend as those at six months. The activity of BG was 20% lower in soils incubated at 30°C than those at 20°C ($P < 0.02$; Figure 2D) and was 16% higher with the addition of kudzu extract ($P = 0.03$; Figure 2E) compared to soils that received water. The substrate addition treatment affected the activity of NAG where soils that received kudzu extract had 20% lower activity than water applied soils ($P = 0.01$; Figure 2F). Unlike the activity at six months, after 14 months of incubation the peroxidase enzyme activity in soil incubated at 30°C was 43% higher compared to soils incubated at 20°C ($P = 0.04$; Figure 2G).

Stable Aggregate Fraction

The mass of stable aggregate fraction was quantified by chemical dispersion of the macro-aggregate fraction in soils harvested at six months (Figure 3). The mass of macroaggregate that remained after dispersion was 13% higher in soils that received kudzu extract than the control soils ($P < 0.01$).



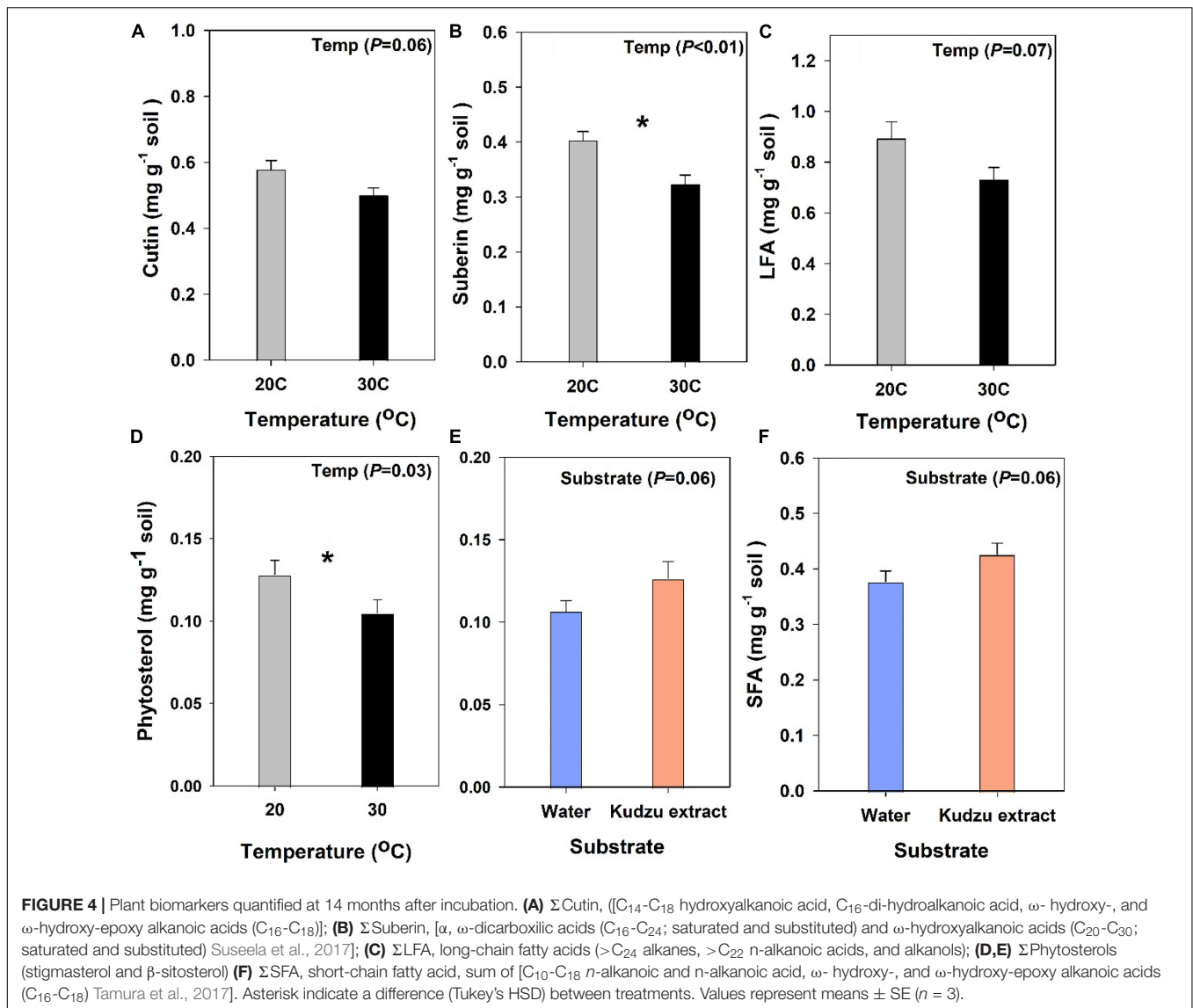


Chemical Composition of Organic Matter

Change in the molecular level composition of organic carbon was compared using the concentration of the extractable plant and microbial derived lipid biomarkers (Figures 4A–F). Among the five different compound classes that were identified, both suberin (19.8%; Figure 4B) and phytosterol (18%; Figure 4D) decreased significantly with higher temperature ($P < 0.05$) while cutin (13.5%; $P = 0.06$; Figure 4A) and LFA (17.4%; $P = 0.07$; Figure 4C) decreased marginally with higher temperature. Soils added with kudzu extract had marginally higher content of phytosterol (20%; Figure 4E) and SFA (17%; $P = 0.06$; Figure 4F) than the control soils. The concentration of lignin derived monomers such as syringyl, vanillyl, and cinnamyl phenols (SVC) were marginally lower (12%) in soils incubated at 30°C compared to soils at 20°C ($P = 0.07$; Figure 5).

Temperature Sensitivity of Soil Carbon Efflux

Temperature sensitivity of the CO_2 efflux (Q_{10}) between the substrate treatments (Figure 6) were compared using the cumulative CO_2 efflux from the two temperatures (eq.



1). The higher Q₁₀ value for kudzu applied soil was only observed during the first 24 h. After one week of the substrate addition (168 h), the Q₁₀ value was stabilized at around 1.4 for soils that received kudzu input. The control (water) samples showed a linear increase of Q₁₀ until 2 weeks after application (336 h) and stabilized at ~ 2.4 at the end of the incubation.

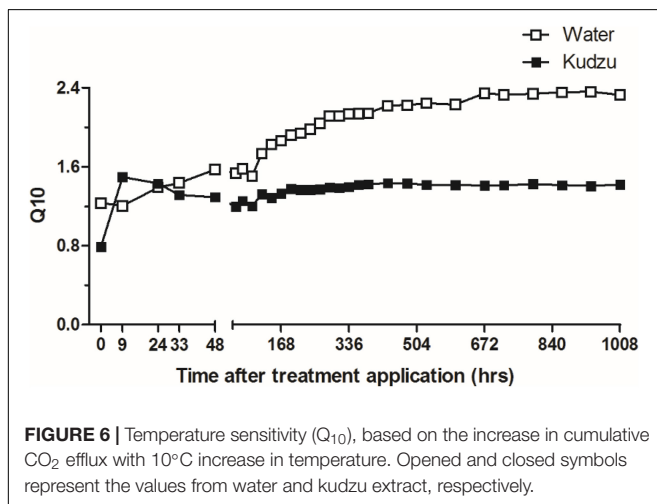
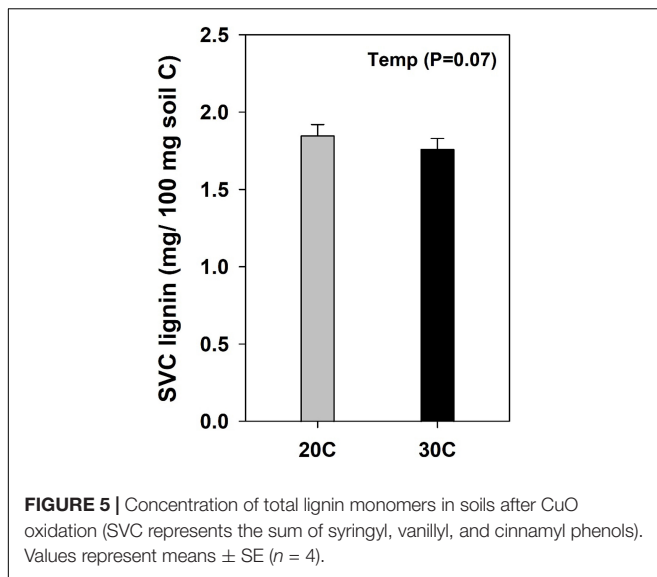
DISCUSSION

Differential Effect of Labile Substrate and Warming on SOC Composition

Warming is projected to increase the catalytic activity of enzymes (Davidson and Janssens, 2006) and decrease the microbial CUE while decomposing substrates of low quality (Conant et al., 2011) leading to the release of soil carbon. Furthermore, the higher input of labile carbon through litter fall promotes microbial

decomposition of native SOM in turn reducing the C storage in invaded soils (Strickland et al., 2010; Tamura and Tharayil, 2014). Thus, combined warming and labile C input could have an additive effect on soil C loss. We hypothesized that the input of labile carbon would instigate a greater soil C loss under elevated temperature than at ambient temperature. However, after 14 months of incubation the addition of labile substrates did not additively decrease soil carbon content. Although field studies have exhibited greater decrease of native carbon content due to labile litter input from invasive species, the observed trend of carbon quantity may have been potentially due to the relatively shorter incubation time of the study (Dungait et al., 2012). Our results exhibited only a trend of decreasing carbon with warming treatment (Supplementary Figure 1) suggesting that a longer duration of incubation may result in significant differences in carbon stocks.

Although warming and substrate addition treatments did not affect the quantity of carbon, our study revealed differences



in microbial community and enzyme activity at different temperatures that potentially affected the composition of SOC. At six months after incubation, an increase in the activity of BG with the addition of kudzu extract was observed only in soils that were incubated at $30^\circ C$ indicating that labile substrate input could alter the temperature response of enzyme activity (Fissore et al., 2013). However, after 14 months of incubation when the soils became more recalcitrant, warming decreased the activity of BG while kudzu addition increased the activity. The addition of labile substrate would increase the soil microbial activity as evident from the higher activity of BG after 14 months of incubation in soils that received kudzu extract (Figure 2B). Similar, higher activity of glucosidase has been reported following the addition of substrates with low C:N ratios (Fog, 1988; Berg and Matzner, 1997; Bastida et al., 2013).

The increase in fungal biomass (ergosterol; Figure 1A) in soils that received kudzu extract, after six months of incubation subsequently increased the activity of NAG (Figure 2C) which

releases N from chitin present in fungal cell walls (Leake and Read, 1990). As fungi are more capable of degrading recalcitrant fraction (plant heteropolymers) than bacteria (Sinsabaugh, 2010), the periodic input of kudzu (labile) substrate with high N may have suppressed the fungal biomass (Figure 1D) resulting in lower NAG activity at 14 months after incubation (Figure 2F). Previous study from the same pine forest site has reported a decrease in fungal biomass when pine soils were invaded by kudzu (Tamura and Tharayil, 2014). Soil peroxidase activity did not vary with the input of kudzu extract at either 6 or 14 months after incubation. However, at 14 months after incubation, the peroxidase activity increased with warming (Figure 2G) leading to a corresponding decrease in the content of lignin (Figure 5). A recent climate warming experiment reported a warming induced shift in fungal community by selecting fungi with a higher capacity to depolymerize recalcitrant carbon (Treseder et al., 2016).

As the initial study soils from pine forest were characterized by higher abundance of cutin, wax and suberin-derived lipids (Tamura and Tharayil, 2014), the molecular biomarker analysis of soils at 14 months after incubation delineated the change in SOC composition with temperature and substrate addition (Figure 4). The biomarker analysis revealed that warming resulted in the loss of plant derived compounds such as cutin, suberin, phytosterol and LFA (Figure 4), which are otherwise slower to decompose and often preserved in soil (von Lützwow et al., 2006). Warming could increase the kinetics of reaction catalyzed by the microbes (Davidson and Janssens, 2006), thus higher response in degradation rate was anticipated for recalcitrant fraction that requires higher activation energy (Suseela et al., 2013). Similar reduction of aliphatic compounds such as cutin and suberin with warming was observed in the field manipulation experiment (Pisani et al., 2014). Moreover, the concentration of lignin monomers was also lower in soils incubated at $30^\circ C$ than $20^\circ C$ (Figure 5) potentially due to the observed higher activity of soil peroxidase (Figure 2G). Recent meta-analysis has also reported increased ligninase activity with warming (Chen et al., 2018). Similar, lower concentration of lignin with warming treatment was observed in previous studies (Feng et al., 2008; Pisani et al., 2014) and greater carbon loss due to warming was reported to be correlated with increased ratios of ligninase to cellulase activity (Chen et al., 2020). Although warming increased the degradation of plant heteropolymers, the input of labile C resulted in the accumulation of microbial products (Cotrufo et al., 2013; Tamura et al., 2017) evident from the marginally higher abundance of short-chain fatty acids (Figure 4F) in soils that received kudzu extract. The increase in SFA could indicate higher concentration of degradation products of plant-derived long-chain fatty acids and/or bacterial biomass (Otto et al., 2005). Overall, the molecular composition of resultant soil delineated the impact of warming in decreasing the abundance of recalcitrant fraction of soil C, while the input of labile extract increased the microbial products which are potentially more stable as it can form organo-mineral associations (Cotrufo et al., 2013, 2015; Tamura et al., 2017; Sokol et al., 2019). These results suggest that future warming and addition of labile substrates may affect soil

carbon by differentially influencing the utilization of carbon compounds of varying lability.

Addition of Labile Substrate Decreased the Temperature Sensitivity of C Mineralization

Soil organic matter is a highly heterogeneous matrix composed of both labile and recalcitrant compounds. Although recalcitrant C has a higher temperature sensitivity (Davidson and Janssens, 2006), microbes could preferentially decompose labile fraction within the soil (Feng et al., 2008; Pisani et al., 2014) or litter matrix (Suseela et al., 2013). Thus, the labile C addition could reduce the temperature sensitivity of the SOC mineralization rate by hindering the decomposition of recalcitrant fraction. For example, the quantity of phytosterol was higher in soils that received kudzu-extract compared to the control (Figure 4E). Since kudzu leaf tissue contained negligible amount of phytosterol (Tamura and Tharayil, 2014), this observation could indicate that the abundance of labile C reduced the degradation of phytosterol. Therefore, decomposition of recalcitrant compounds could be hindered by labile C (kudzu-extract), and overall temperature sensitivity could be consequently lower for soils that received labile extract.

We observed a lower temperature coefficient (Q_{10}) for the kudzu added soil compared to the control soils (Figure 6). The immediate increase in Q_{10} after the labile substrate input is often observed due to the activity of fast-growing bacteria responding to increased labile nutrient source, that diminish within the first 24 h (Dungait et al., 2013; Thiessen et al., 2013). The rate of carbon mineralization through microbial decomposition of SOC is influenced by the quality of the substrates, and the accessibility of microbes to the substrates (von Lützow et al., 2006). Soils that received kudzu substrate had greater macro-aggregate stability compared to the control (Figure 3). The enhancement of aggregate stability and reduced SOM mineralization rate (Park et al., 2007) and subsequent lower temperature sensitivity with the input of labile C was also reported previously (Qin et al., 2019). Therefore, these results suggest that labile substrate input could promote physical protection of the SOC that partly would have contributed to a lower temperature sensitivity of CO_2 efflux.

CONCLUSION

Climate warming and invasion of exotic plant species are two major global change factors that threaten the sustainability of native ecosystems. Our study examined the interactive effects

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of warming and labile substrate input by invasive plants on the quantity and composition of native SOC through changes in microbial biomass and enzyme activity. Although the predicted additive impact of warming and input of labile C in reducing soil C was not evident, the study demonstrated that the input of labile C increased aggregate stability, abundance of short chain fatty acids and decreased the temperature sensitivity of soil C efflux. Conversely, warming increased peroxidase activity and altered the soil carbon composition by decreasing the abundance of recalcitrant plant polymers such as cutin, suberin and lignin consistent with the kinetics theory. The decomposition of chemically complex and recalcitrant carbon would increase the soil carbon efflux and have the potential to accelerate warming. Our results thus indicate that the input of labile C from invader biomass and warmer climates could affect microbial activity and the molecular-level soil carbon composition which could alter SOC storage in invaded ecosystems.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

VS and MT conceived and designed the study. MT conducted the experiment and wrote the draft of the manuscript with input from VS. Both authors approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/ffgc.2021.691302/full#supplementary-material>

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