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Microbial regulation of soil organic matter decomposition at the regional scale

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1 Summary

The fate of soil organic carbon (SOC) is one of the greatest uncertainties in predicting future climate. Soil microorganisms, as primary decomposers of SOC, control C storage in terrestrial ecosystems by mediating feedbacks to climate change. Even small changes in microbial SOC decomposition rates at the regional scale have the potential to alter land-atmospheric feedbacks at the global scale. Despite their critical role, the ways in which soil microorganisms may change their abundances and functions in response to the climate change drivers of soil temperature and moisture is unclear. Additionally, most existing C models do not consider soil microorganisms explicitly as drivers of decomposition, one consequence of which is large variability in predicted SOC stock projections. This demonstrates the need for a better mechanistic understanding of microbial SOC decomposition at large scales. This thesis was designed to clarify the role of microbial SOC decomposition dynamics in response to climate change factors in two geographically distinct areas and land-use types. The hypothesis was that microbial communities would be adapted to climatic and edaphic conditions specific to each area and to the SOC organic quality in each land-use and would therefore exhibit distinct responses to soil temperature and moisture variations.

Three studies were performed to address the goals of this thesis. The first study aimed to clarify temporal patterns of degradation in C pools that varied in complexity by modelling *in situ* potentials of microbially produced extracellular enzymes. Temperature and moisture sensitivity patterns of C cycling enzymes were followed over a period of thirteen months. The second study investigated group-specific temperature responses of bacteria and fungi to substrate quality variations through an additional incubation experiment. Here, complex environments were mimicked in order to determine the dependence of microbial responses not only on environmental conditions, but also under conditions of inter- and intra-specific community competition. Changes in microbial community composition, abundance, and function were determined at coarse (phospholipid fatty acid – PLFA, ergosterol) and relatively fine resolutions (16S rRNA, taxa-specific quantitative PCR, fungal ITS fragment). A third study investigated 1) the spatial variability of temperature sensitivity of microbial processes, and 2) the scale-specificity and relative significance of their biotic and physicochemical controls at landscape (two individual areas, each ca. 27 km^2) and regional scales (pooled data of two areas).

Strong seasonal dependency was observed in the temperature sensitivities (Q_{10}) of hydrolytic and oxidative enzymes, whereas moisture sensitivity of β-glucosidase activities remained stable over the year. The range of measured enzyme Q_{10} values was similar irrespective of spatial scale, indicating a consistency of temperature sensitivities of these enzymes at large scales. Enzymes catalyzing the recalcitrant SOC pool exhibited higher temperature sensitivities than enzymes catalyzing the labile pool; because the recalcitrant C pool is relatively large, this could be important for understanding SOC sensitivity to predicted global warming. Response functions were used to model temperature-based and temperature and moisture-based *in situ* enzyme potentials to characterize seasonal variations in SOC decomposition. *In situ* enzyme potential explained measured soil respiration fluxes more efficiently than the commonly used temperature-respiration function, supporting the validity of our chosen modelling approach. As shown in the incubation experiment, increasing temperature stimulated respiration but decreased the total biomass of bacteria and fungi irrespective of substrate complexity, indicating strong stress responses by both over short time scales. This response did not differ between study areas and land-uses, indicating a dominant role of temperature and substrate quality in controlling microbial SOC decomposition. Temperature strongly influenced the responses of microbial groups exhibiting different life strategies under varying substrate quality availability; with soil warming, the abundance of oligotrophs (fungi and gram-positive bacteria) decreased, whereas copiotrophs (gram-negative) increased under labile C substrate conditions. Such an interactive effect of soil temperature and substrate quality was also visible at the taxon level, where copiotrophic bacteria were associated with labile C substrates and oligotrophic bacteria with recalcitrant substrates. Which physicochemical and biological factors might explain the observed alterations in microbial communities and their functions in response to climate change drivers at the regional scale was investigated in the third study. Here, it was shown that the soil C:N ratio exerted scale-dependent control over soil basal respiration, whereas microbial biomass explained soil basal respiration independent of spatial scale. Factors explaining the temperature sensitivity of soil respiration also differed by spatial scale; extractable organic C and soil pH were important only at the landscape scale, whereas soil texture as a control was independent of spatial scale.

In conclusion, this thesis provides an enhanced understanding of the response of microbial C dynamics to climate change at large scales by combining field measurements with innovative laboratory assays and modelling tools. Component specific degradation rates of SOC using

extracellular enzyme measurements as a proxy, group-specific temperature sensitivities of microbial key players, and the demonstrated scale-specificity of factors controlling microbial processes could potentially improve the predictive power of currently available C models at regional scale.

2 Zusammenfassung

Der Verbleib und das Verhalten von bodenbürtigem organischen Kohlenstoff (SOC) bergen mit die größten Unsicherheiten bei der Vorhersage des zukünftigen Klimas. Als primäre Zersetzer von SOC kontrollieren Bodenmikroorganismen die Speicherung von Kohlenstoff in terrestrischen Ökosystemen, da sie die mediatorische Rückkopplung zum Klimawandel darstellen. Schon geringe Änderungen der SOC-Abbauraten auf regionaler Skala haben das Potential, die Land-Atmosphären-Rückkopplungen auf globaler Ebene zu beeinflussen. Trotz ihrer wichtigen Rolle ist unklar, wie Bodenmikroorganismen ihre Abundanz und Funktion an Klimawandel, Bodentemperatur und -feuchte anpassen. Darüber hinaus werden Bodenmikroorganismen in den meisten C-Modellen nicht explizit als bestimmende Komponente des Kohlenstoffabbaus einbezogen, was in einer großen Variation der vorhergesagten C-Vorräte resultiert. Dies unterstreicht die Notwendigkeit eines besseren mechanistischen Verständnisses des mikrobiellen SOC-Abbaus auf großen Skalen. Die vorliegende Dissertation wurde ausgeführt, um die Rolle der mikrobiellen SOC-Abbaudynamik in Anhängigkeit von Klimawandelfaktoren in zwei geographisch getrennten Regionen und Landnutzungstypen zu klären. Die Hypothese lautete, dass mikrobielle Gemeinschaften sich an die regional spezifischen klimatischen und edaphischen Bedingungen sowie an die Qualität des SOC in jeder Landnutzungsform anpassen und daher unterschiedliche Reaktionen auf Bodentemperatur- und -feuchtigkeitsvariationen zeigen.

Um das Ziel dieser Dissertation zu erreichen, wurden drei Studien durchgeführt. Die erste Studie zielte darauf ab, den zeitlichen Verlauf des Abbaus von unterschiedlich komplexen C-Quellen durch die Modellierung des *in situ*-Potentials von mikrobiellen, extrazellulären Enzymen zu untersuchen. Die Temperatur- und Feuchtesensitivität von Enzymen des Kohlenstoffkreislaufs wurde über einen Zeitraum von dreizehn Monaten beobachtet. Die zweite Studie untersuchte gruppenspezifische Temperaturabhängigkeiten von Bakterien und Pilzen im Hinblick auf Substrate unterschiedlicher Qualität in einem separaten Inkubationsexperiment. Hierbei wurden komplexe Umweltbedingungen nachgestellt, um die Abhängigkeit mikrobieller Reaktionen nicht nur von Umwelteigenschaften, sondern auch von inter- und intraspezifischer Gemeinschaftskonkurrenz zu bestimmen. Veränderungen in der mikrobiellen Gemeinschaftsstruktur, Abundanz und Funktion wurden mittels grober (Phospholipidfettsäuren – PLFA, Ergosterol) und relativ feiner Auflösung (16S rRNA, taxaspezifische quantitative PCR, pilzliche ITS-Fragment) bestimmt. Eine dritte

Studie untersuchte 1) die räumliche Variabilität der Temperatursensitivität mikrobieller Prozesse, und 2) die skalenspezifische und relative Bedeutung ihrer biotischen und physikalisch-chemischen Steuerungsgrößen auf Landschafts- (zwei individuelle Regionen, jede 27 km²) und Regionalskala (zusammengefasste Daten zweier Regionen).

Es wurden starke jahreszeitliche Abhängigkeiten der Temperatursensitivität (Q_{10}) von hydrolytischen und oxidativen Enzymen beobachtet, während die Feuchtigkeitssensitivität der β-Glucosidaseaktivitäten im Jahresverlauf stabil blieb. Der Bereich der gemessenen Q_{10}-Werte variierte, unabhängig von der räumlichen Skala, wenig, was auf eine konstante Temperatursensitivität dieser Enzyme auf größerer räumlicher Skala hinweist. Enzyme, die in den Abbau von rekalzitrantem SOC involviert sind, wiesen eine höhere Temperatursensitivität verglichen mit solchen Enzymen auf, die labilere Verbindungen abbauen. Da der Anteil rekalzitranter C-Quellen im Boden relativ groß ist, könnte dies wichtig sein, um die SOC-Sensitivität auf den vorhergesagten Klimawandel zu verstehen. Um die temperaturbasierten sowie temperatur- feuchtigkeitsbasierten *in situ*-Enzympotentiale zur Charakterisierung von saisonalen Variationen im SOC-Abbau zu modellieren, wurden Antwortfunktionen genutzt. *In situ*-Enzympotentiale erklärten die gemessenen Bodenatmungsflüsse effektiver als die üblich genutzte Temperatur-Atmungs-Funktion. Dies unterstrich die Validität des gewählten Modellierungsansatzes. Wie im Inkubationsexperiment gezeigt wurde, erhöhte steigende Temperatur die Atmung, aber verringerte die Gesamtbiomasse von Bakterien und Pilzen unabhängig von der Substratkomplexität. Dies deutet auf starke Stressreaktionen beider Gruppen innerhalb kurzer Zeiträume hin. Die Reaktion unterschied sich nicht zwischen den Untersuchungsregionen oder Landnutzungen, was auf die dominierende Rolle von Temperatur und Substratqualität bei der Steuerung des mikrobiellen SOC-Abbaus hindeutet. Die Temperatur hatte unter variierender Substratqualitätsverfügbarkeit starken Einfluss auf die mikrobiellen Gruppen mit unterschiedlichen Lebensstrategien: bei Vorhandensein labiler C-Substrate nahm die Abundanz von Oligotrophen Mikroorganismen (Pilze, Gram positive Bakterien) mit der Bodenerwärmung ab, während Kopiotrophe (Gram negative Baktieren) zunahmen. Solch ein interaktiver Effekt von Bodentemperatur und Substratqualität war auch auf der Taxonebene sichtbar, wo Kopiotrophe Bakterien stärker mit labilen und Oligotrophe Bakterien stärker mit rekalzitranten C-Substraten assoziiert waren. Welche physikalisch-chemischen und biologischen Faktoren die beobachteten Veränderungen in mikrobiellen Gemeinschaften sowie ihre Funktionen

in Reaktion auf Einflussfaktoren des Klimawandels auf regionaler Skala erklären, wurde in der dritten Studie untersucht. Hier konnte gezeigt werden, dass das C:N-Verhältnis des Bodens einen skalenabhängigen Einfluss auf die Basalatmung des Bodens hatte, während die mikrobielle Biomasse die Basalatmung unabhängig von der räumlichen Skala erklärte. Faktoren, die die Temperatursensitivität der Bodenatmung erklärten, unterschieden sich ebenfalls zwischen den räumlichen Skalen: der extrahierbarer organischer C und der Boden pH-Wert waren nur auf der Landschaftsskala relevant, während die Bodenart als Steuerungsgröße unabhängig von der räumlichen Skala war.

Schlussfolgernd bietet diese Dissertation ein verbessertes Verständnis für die Reaktionen von mikrobiellen C-Dynamiken auf den Klimawandel auf größeren Skalen, in dem sie Feldmessungen mit innovativen Laboranalysen und Modellierungswerkzeugen verbindet. Komponentenspezifische Abbauraten von SOC, die extrazelluläre Enzymmessungen als Stellvertretervariable, gruppenspezifische Temperaturabhängigkeiten mikrobieller Hauptakteure und die demonstrierte Skalenspezifität von Einflussgrößen auf mikrobielle Prozesse nutzen, haben das Potential die Vorhersagekraft aktuell verfügbarer C-Modelle auf der regionalen Skala zu verbessern.

3 General Introduction

3.1 Soils at the core of C cycling; the context of climate change

The global soil carbon stock is approximately 1500 Pg in the top 100 cm, making the soil carbon stock bigger than both atmospheric (1.7 times) and vegetation (~ 2.7 times) stocks (Lal, 2018). Carbon (C) storage in soil systems is regulated by the balance of C inputs, its stabilization, and its losses (Yigini and Panagos, 2016). Soil organic carbon (SOC) is an extremely complex mixture that originates from many biotic and abiotic sources including, but not limited to, partially decomposed plant and animal residues and microbial biomass (Balser, 2005; Conant et al., 2011; Schmidt et al., 2011). Carbon exits the soil system primarily through soil respiration, including both autotrophic (by roots) and heterotrophic (by microorganisms) respiration (Schlesinger and Andrews, 2000). Other processes through which C is lost from soils include lateral fluxes; for instance, dissolved C export through crop harvest or runoff (Guenet et al., 2018).

Soil harbours an enormous diversity of life and its inhabitants consist of populations of macrofauna, mesofauna, microfauna and microflora (Nannipieri et al., 2003). Soil microorganisms, as primary decomposers of SOC, mediate the size of the SOC pool by contributing up to 90 % of process reactions, hence playing a key role in governing C cycling (Nannipieri et al., 2003; Balser and Wixon, 2009; Portillo et al., 2013; Vries and Shade, 2013). Microorganisms benefit soil ecosystems in many ways; for example, enhancing biodiversity and productivity of above ground plant biomass by increasing nutrient availability for their growth, by scavenging contaminants (e.g. heavy metals and pesticides), and by increasing resilience and resistance to different environmental stresses (Poll et al., 2010; Lukac et al., 2017; Lladó et al., 2018). Besides providing the above mentioned ecosystem services, soil microorganisms also structural complexity of SOC through depolymerization and by synthesizing metabolites leading to aggregate formation, hence SOC stabilization (Chotte, 2005; Conant et al., 2011). Various factors exert control over SOC dynamics through control of microbial growth and respiration rates. These include intra- and inter-microbial community competition, e.g., for available substrate and for sound habitat through antibiotic production (Fontaine et al., 2003; Revilla-Guarinos et al., 2014), SOC quality / quantity, soil temperature, soil moisture (controlling, e.g., substrate / O_2 / microbial diffusion) (Davidson and Janssens, 2006; German et al., 2011; Steinweg et al., 2012; Allison et al., 2014), and other physicochemical soil properties including soil texture, exchangeable calcium and iron- and

aluminium-oxyhydroxides, pH, and soil C:N ratio (Ding et al., 2014; Min et al., 2014; Spohn, 2015; Rasmussen et al., 2018).

Human activities influence the earth's energy balance by altering atmospheric concentrations of radiatively vital greenhouse gases, such as CO_2, contributing significantly to the warming of earth's mean surface temperature (Cubasch et al., 2013). Observations have shown an increase of 0.85 °C in mean global surface temperature over the period 1880 – 2012 and a further increase, at a great rate, is expected by the end of 2100 (IPCC, 2014). On the basis of different climate change scenarios, climate warming may increase earth's mean surface temperature to as high as 4.8 °C by the end of the twenty-first century (relative to 1986 – 2005) (IPCC, 2014). Rising atmospheric CO_2 concentrations and the resulting climate change are projected to increase primary productivity and hence litter input to SOC pools during the next century, thus strengthening the negative feedback. However, soil warming is also projected to accelerate SOC decomposition, which like any other kinetic process, reacts strongly positively to a temperature increase (Conant et al., 2011; Guenet et al., 2018). Therefore, given the magnitude of the soil C stock, clarification of the SOC decomposition response to predicted global climate change is central to understanding dynamics of the coupled carbon-climate system (Ajwa and Tabatabai, 1994; Schlesinger and Andrews, 2000).

3.2 Sensitivity of SOC decomposition in the context of climate change

A great deal of existing literature focuses on understanding and clarifying the dynamics of SOC under a changing climate (Trumbore, 1997; Zogg et al., 1997; Davidson et al., 1998; Davidson and Janssens, 2006; Fierer et al., 2006; Poll et al., 2013; Tang and Riley, 2015; Klimek et al., 2016). Still, the fate of SOC is uncertain and an important element of this uncertainty is the response of SOC quality to climate change. Plant growth will be altered as a result of global climate change. CO_2 enrichment / fertilization effect, rising temperatures, modified specific leaf area, and plant C allocation patterns – all can alter input of detritus in both absolute (litter quantity) and relative terms (litter quality) (Cha et al., 2017; Chen and Chen, 2018). Uncertainty of the response of SOC to climate warming is often discussed in terms of SOC structural complexity, i.e., the rate and extent of decomposition of labile and complex SOC pools (Xu et al., 2012; Luo et al., 2017a). Like any other biochemical reaction, SOC decomposition is inherently sensitive to temperature, and decomposition rates may increase with increasing temperature, which is in agreement with the

Arrhenius theory (Sierra, 2012). Temperature sensitivity of SOC decomposition is commonly quantified as the Q_{10} value; it represents the rate of change in basal respiration rate for every 10 °C difference in soil temperature (Davidson and Janssens, 2006; Tang et al., 2017). Over the last decades, many studies have supported the temperature sensitivity of SOC decomposition with the "C quality temperature" hypothesis, which states: reactions with high activation energies (E_a, representing recalcitrant C pool) have higher temperature sensitivity (high Q_{10} values) than reactions with low E_a (low Q_{10} values, representing labile C pool) (Fierer et al., 2003; Knorr et al., 2005; Davidson and Janssens, 2006; Vanhala et al., 2007; Conant et al., 2008; Klimek et al., 2016). However, studies have also found evidence that contradicts this hypothesis, i.e., an inverse relationship (Benbi et al., 2014) or even no difference in Q_{10} values between SOC pools of different qualities (Fang et al., 2005; Conen et al., 2006; Fang et al., 2006). Higher sensitivity of "physio-chemical SOC stabilization processes" (see below) than "SOC respiration reactions" may be responsible for these contradictory findings (Thornley and Cannell, 2001). It may also be that much of the SOC persisting in soil is of high quality, but due to direct or indirect interactions with mineral surfaces and with other soil organic matter, it is either insoluble or spatially inaccessible to decomposers, resulting in an altered temperature-related response (Colman and Schimel, 2013). Sierra (2012) attributed the inconsistent temperature sensitivities of SOC pools to different measures used to explain it, such as turnover times, activation energies, qCO_2 and Q_{10}. Therefore, clear understanding of the response of SOC dynamics to warming climate remains an unresolved issue.

Assignment of soil organic C into different pools and fractions, for estimation of absolute pool size with individual turnover rates, has been carried out using different techniques. For instance, SOC can be physically separated into aggregate, particle size, and density fractions; or can be fractionated using various wet chemical procedures; high-gradient magnetic separation (HGMS), nuclear magnetic resonance (NMR) spectroscopy, diffuse reflectance Fourier transform mid-infrared spectroscopy (midDRIFTS), and their combinations (Lützow et al., 2007; Kunlanit et al., 2014). Each fractionation method has advantages and disadvantages; for instance, sample preparation and measurement time, method accuracy and cost effectiveness. Recently, midDRIFTS has been used successfully to characterize SOC at the regional scale (Mirzaeitalarposhti et al., 2015) highlighting the usefulness of this technique in time and perhaps cost effectiveness.

Discrepancies in SOC decomposition responses to temperature, besides above explained SOC quality aspect, may be directly or indirectly related to other physicochemical drivers, such as interactive effects of CO_2 production and nutrient availability (N, P), soil C:N ratio, and pH (Fierer et al., 2003; Klimek et al., 2016; Craine et al., 2010; Schmidt et al., 2011; Min et al., 2014). Soil texture controls temperature-related SOC decomposition by, for example, a) physical protection, in which SOC becomes occluded within soil aggregates; b) chemical protection, in which SOC becomes adsorbed onto mineral surfaces through strong covalent and electrostatic bonds; and by c) altering soil water holding capacity and solution chemistry through pH buffering (Sollins et al., 1996; Thornley and Cannell, 2001; Davidson and Janssens, 2006). The temperature sensitivity of soil respiration is also controlled by soil moisture, another important factor which will be affected by predicted climate change. For example, altered soil water films control substrate diffusion, and diffusion of microbial decomposers (Davidson and Janssens, 2006). Variations in soil moisture have been associated with abrupt changes in soil respiration patterns with different mechanisms responsible, including disruption of aggregates and subsequent release of physically protected SOC, microbial cell lysis and / or release of their osmoregulatory solutes to soil that could be further mineralized by microbial biomass (Fierer and Schimel, 2003). Allision and Treseder (2008) suggested that rising temperatures may not result in a positive feedback to climate change if soil moisture remains a limiting factor in SOC decomposition.

Seasonal factors, such as variation in soil temperature and microbial community composition, may also modify the temperature sensitivity of microbial processes. For example, sensitivity of SOC decomposition is greater at low than at high temperatures and strongly deviates from an assumption of constant Q_{10} across temperatures (Koch et al., 2007; Kirschbaum, 2010). Different studies provide complementary evidence for this: regions with cold-climate and high elevations (cool temperature) exhibited greater temperature sensitivity than regions with warm-climate and low elevation gradients (warm temperature) (Schindlbacher et al., 2010).

3.3 Microbial decomposition of SOC in the context of climate change

Soil microorganisms play a central role in soil's service of maintaining biogeochemical cycles by processing virtually all types of soil organic matter; from xenobiotics to naturally occurring polyphenols and related compounds (Nannipieri et al., 2003). Soil microbial biomass consists of populations of organisms that differ from each other in many respects; for example, life history

traits, thermal tolerances, and dispersal ability (Classen et al., 2015). Soil bacteria and fungi, comprising > 90 % of soil microbial biomass, are the major drivers of organic C decomposition (Jagadamma et al., 2014). Abundances and functions of soil microorganisms may be affected directly or indirectly by the same major factors driving climate change. For example, climate warming and associated soil moisture dynamics may alter soil microbial community composition and its plasticity and resilience under environmental stresses directly (Schimel et al., 2007; Allison and Treseder, 2008; Griffiths and Philippot, 2013; Morrissey et al., 2017) or indirectly, through altered plant community composition, litter quality and altered plant root phenology and exudation (Badri and Vivanco, 2009; Drake et al., 2013; Abramoff and Finzi, 2014; Robinson et al., 2018).

Soil microorganisms respond to a warming climate by shifting metabolic processes and rates; however, discrepancies in our understating still exist as to the direction of the responses, and possible reasons for this could be microbial adaptation (Bradford, 2013). While most soil warming experiments, in lab- and field-scale studies and at short time scale (days to weeks and over several months), have shown an increase in SOC decomposition with increasing temperature, studies carried out over longer time periods (several years) highlight the limitations and complex interactions between temperature, substrate quality, and its availability to microbial decomposer communities in controlling decomposition rates (Burke et al., 2003). For example, a decrease in soil respiration rates in soil warming experiments to pre-warming levels after an initial short-lived increase have been associated with depletion of labile SOC, microbial biomass, and their combination, as well as acclimation of microbial communities to warming with resulting adjustments in their C use efficiency (CUE) patterns (Knorr et al., 2005; Hartley et al., 2007; Bradford et al., 2008; Lützow and Kögel-Knabner, 2009a; Allison et al., 2010; Birge et al., 2015). Classical understanding suggests that fungi decompose recalcitrant substrates while bacteria usually decompose labile substrates (Kramer et al., 2016). Similarly, the fungal to bacterial ratio in soil is associated with C sequestration potential, in which greater fungal abundance is usually associated with greater C storage. Therefore, shifts in soil microbial substrate utilization patterns may critically alter the rate of C loss from soils (Six et al., 2006). However, a fungal association with only recalcitrant substrate decomposition has recently been challenged (Kramer et al., 2016; Müller et al., 2017). CUE, the proportion of assimilated C utilized for growth and enzyme production vs. that utilized for maintenance, is a function of both C quality and the degradative efficacy of microbial community. For example, fungi are more efficient users than soil bacteria of

the C they take up (Cotrufo et al., 2013; Frey et al., 2013; Sinsabaugh et al., 2016). Frey *et al.,* (2013) observed that temperature controls microbial CUE patterns of different SOC fractions; recalcitrant substrate utilization efficiency was reduced as temperature increased. However, Frey *et al.,* (2013) also observed that microbes adapted their CUE with continuous soil warming that resulted in a weaker decline in CUE of recalcitrant substrate with increasing temperature.

Knowledge of the temperature-sensitive responses of microbial abundances and activities and of specific microbial functional groups within a given community will also improve our understanding of microbial SOC decomposition (Malcolm et al., 2008; Classen et al., 2015). Soil temperature has been shown to influence microbial community abundance and composition (Zogg et al., 1997; Waldrop and Firestone, 2004). The temperature response of microbial SOC decomposition may in fact be tightly linked to microbial life strategies. Copiotrophic microorganisms have high nutritional requirements, thrive in high quality substrate environments (labile) and exhibit rapid growth rates under abundant resource availability. In contrast, oligotrophic microorganisms grow relatively slowly and exploit low nutrient environments (Fierer et al., 2007). Bai *et al.,* (2017b) found that copiotrophs were positively correlated with the temperature sensitivity of SOC decomposition whereas oligotrophs were negatively correlated; it follows that soils dominated by copiotrophs may respond strongly to climate warming. There is compelling evidence that soil microorganisms vary in their responses to temperature alterations in two aspects, 1) difference with respect to organisms – fungi perform better at low temperatures, while bacteria benefit more from warm temperatures (Pietikäinen et al., 2005; Bell et al., 2008; Bell et al., 2009), and 2) difference with respect to regional adaptation – cold adapted microbial communities may be more temperature sensitivities than warm adapted microbial communities (Bradford et al., 2008; Wei et al., 2014). This temperature sensitive response could also be observed at the community level, for example, as has been observed for ectomycorrhizal fungi, between ectomycorrhizal and saprotrophic fungi and between gram-positive and gram-negative bacteria (Allison and Treseder, 2008; Bell et al., 2009; Malcolm et al., 2008). Additionally at the taxon level, *Proteobacteria* and *Bacteroidetes* may exhibit negative relation to increasing temperature, whereas *Acidobacteria, Verrucomicrobia* and *Firmicutes* abundances may increase with soil warming (DeAngelis et al., 2015; Mateos-Rivera et al., 2016; Bai et al., 2017b).

Temperature changes are often coupled with altered soil moisture content. But studies focusing on only single factor, for example temperature, may under- or over-estimate the response of microbial communities to given environmental alterations (Steinweg et al., 2012; Classen et al., 2015). Changes in soil water potentials may significantly affect microbial community composition and functions depending on those organisms' physiology. For example, fungi are characterized by higher tolerance to water fluctuations than bacteria, and within the bacterial community, gram-positive bacteria may resist water stress more efficiently than gram-negative bacteria (Lützow et al., 2006; Schimel et al., 2007; Bell et al., 2009). The moisture sensitivity of microbial communities has also been associated with C sequestration potential; physiological stress caused by moisture reduction potentially reduces microbial C mineralization rates that would otherwise sequester soil C over time (Fierer and Schimel, 2003).

3.4 Role of microbial extracellular enzymes

Soil microorganisms control global C fluxes, nutrient cycling, plant productivity, and atmospheric composition by mineralizing soil organic matter; microbes do this by producing myriad extracellular enzymes (EEs) that deconstruct plant and microbial cell walls and reduce macromolecules to soluble substrates that are microbially assimilable (Schimel and Weintraub, 2003; Sinsabaugh et al., 2008; Baldrian, 2014). Therefore, production and activity of EEs is directly linked to ecosystem function (Wallenstein and Burns, 2011). Important enzymes involved in the cycling of different compounds are: β-glucodidases, endo-cellulases, cellobiohydrolases, and phenol- and per-oxidases involved in C mineralization, amidase asparaginase, urease, and dipeptidase involved in N mineralization, and phosphomonoesterase and phosphodiesterase involved in P mineralization (Kandeler, 1990; Caldwell, 2005; Kandeler et al., 2011; Bünemann, 2015). These enzymes can be broadly categorized into hydrolytic and oxidative enzymes. Hydrolytic enzymes are substrate-specific and catalyze reactions that cleave specific bonds, for instance C–O and C–N bonds. In contrast, oxidative enzymes are less substrate-specific and catalyze reactions that share similar bonds, for instance C–C and C–O–C, using either oxygen or hydrogen peroxide as electron acceptors (Allison et al., 2007; Wallenstein and Burns, 2011). Microbial enzyme production is directly controlled by substrate and enzyme-catalyzed-product concentration; producing enzymes is energetically expensive and requires large quantities of N;

therefore, microbes produce enzymes only when investment costs are lower than the energy retrieved from assimilable products (Allison, 2005; Allison et al., 2014).

Since microbial decomposition of SOM accounts for more than 50 % of the CO_2 flux from soils, and since extracellular enzymes perform the rate limiting steps of microbial SOM decomposition and also reflect changes in microbial communities that decompose SOC of different qualities (see above), understanding the potential of extracellular enzymes would help to clarify the fate of SOC in predicted climate change scenarios (Frey et al., 2013; German et al., 2012). However, interpreting EEs' activity measurements as indices of microbial SOC decomposition is challenging due to many confounding factors, such as the temporal and spatial variability in EE potentials (Baldrian, 2014). A good example of the problem is the influence of temperature on enzyme activities. Studies have demonstrated that EEs have distinct temperature sensitivities that vary between seasons and soil types, within one region or between regions with contrasting organic matter quantities (Koch et al., 2007; Trasar-Cepeda et al., 2007). Seasonal variation in enzyme temperature sensitivity has been associated with isoenzymes possessing distinct temperature sensitivities and also with alterations in plant C inputs and nutrient availability over time (Wallenstein et al., 2009; Bell et al., 2010). Temperature sensitivity of enzyme activities may also be region-specific; enzymes from cold regions may exhibit greater temperature sensitivity than enzymes from warm regions (German et al., 2012) and this differentiation has been associated with the flexibility of enzyme reactive sites to temperature variations and the thermal adaptation of enzyme-producing microbial communities (Bradford, 2013; Wallenstein et al., 2011). Allison *et al.,* (2018) found contradictory results, however, observing warm-climate adapted enzymes which exhibited high temperature sensitivities. Furthermore, EEs catalyzing substrates of varying complexities may also have different temperature sensitivities. For example, Wang *et al.,* (2012) found greater temperature sensitivity in enzymes catalyzing recalcitrant compounds compared to enzymes catalyzing labile C substrate. However, there is disagreement about this finding (Blagodatskaya et al., 2016). Therefore, information about the temperature sensitivity of enzymes' depolymerization of different SOC fractions may improve our understanding of the relative decay rates of different SOC pools (Koch et al., 2007; Davidson and Janssens, 2006).

Temperature sensitivity of EE activities may also be controlled by other factors that vary between regions; for example, soil pH, C:N ratio, soil texture, particle size distribution, and soil moisture

content (Allison et al., 2007; Allison et al., 2018; Burns et al., 2013; Davidson and Janssens, 2006). Min *et al.,* (2014) identified a significant role of pH in controlling temperature sensitivity of C-acquiring enzymes, while no pH-dependent changes were observed in the temperature sensitivity of N-acquiring enzymes. Physical or chemical separation of enzymes from substrates may also alter their apparent temperature sensitivities and produce temperature-sensitive responses much lower than the intrinsic response (Davidson and Janssens, 2006). What makes the temperature-sensitivity of enzymes' response even more complicated, and where little information is available, is fluctuation in soil moisture content and its control over enzyme activities. Reduced soil moisture content affects enzyme diffusion to substrate hindering the formation of enzyme-substrate complexes, and consequently, enzyme temperature dependence (Davidson and Janssens, 2006; Steinweg et al., 2012). However, high diffusion rates are also problematic; they reduce the return on investment for enzyme producing microbes, resulting in enzyme downregulation (Allison et al., 2007). Enzyme downregulation may also be a microbial strategy for coping with water stress; under this conditions, microbes can change their resource allocation from enzyme production to, e.g., osmolyte production (Schimel et al., 2007) leading to an overall reduction in the enzyme temperature sensitivity response. However, slower enzyme turnover rates in dry soils could help enzymes retain activity over a longer time period (Burns et al., 2013). Therefore, in the context of climate warming, understanding these complex interactions of abiotic soil properties and their relative control over enzyme temperature sensitivity may also improve our understanding of soil and ecosystem services (Allison et al., 2018; Henry, 2013).

Associating potential enzyme activities with ecosystem services and extrapolating enzyme temperature- and moisture-based variations to field conditions is a challenging task (Weedon et al., 2011; Steinweg et al., 2012; Burns et al., 2013). Over the last decades, assays of EE have become an increasingly common tool for examining soil microbial responses to climate warming, but these methods are not universal, and consequently make it difficult to compare results from different studies (Burns et al., 2013; Henry, 2013). Current enzyme assays are performed under controlled lab conditions; optimal substrate concentration, pH, and lack of diffusion limitations (Poll et al., 2008) and permit measurement of specific enzyme activities. However, they do not make possible the identification of specific microbial organisms responsible for this activity, e.g., who produces these enzymes (Nannipieri et al., 2003). However, it is well established that lab-based studies

cannot truly represent the interactive controls, for example, of soil temperature and moisture over EE activities (Wallenstein et al., 2011).

3.5 Microbial SOC decomposition and C modelling: the scale issue

The spatial distribution of SOC in soil and its subsequent microbial decomposition provides a basis for understanding C cycling within single hot spots, for example, at the mm scale (Poll et al., 2006). However, decomposition of SOC (hence soil respiration) and differing microbial responses to temperature (influencing microbial abundance and function) vary at temporal and spatial scales larger than the mm scale. For example, soil respiration has been found to vary from less than one meter to landscape (100+ meters) and even continental scales (Martin and Bolstad, 2009; Colman and Schimel, 2013). Spatial variation in the temperature sensitivity of SOC decomposition also exists at the biome scale, especially in boreal and temperate biomes, and from the continental to the global scale (Chen and Tian, 2005; Fierer et al., 2006; Zhou et al., 2009). Regional scale variation in microbial community structure and function has also been reported, and been attributed to different land-use types and soil edaphic properties (Kaiser et al., 2016). Large variations in Q_{10} values of SOC decomposition are also temporal occurring on seasonal scales (Janssens and Pilegaard, 2003). However, it is not clear which factors controlling microbial growth and respiration response to environmental changes (such as temperature) are important at which scale. For example, biogeography, climate and abiotic conditions may shape microbial community abundance and function at the regional scale, physicochemical soil properties and plant communities may influence microbial community structure at centimeter to meter scales, and plant root exudates and soil heterogeneity may be significant at finer scales (Lladó et al., 2018). Despite the huge variation observed in the temperature sensitivity of SOC decomposition, and the fact that the temperature sensitivity of microbial respiration often decreases with increasing temperature, Q_{10} is generally treated as a constant of 2 in C dynamics models (Chen and Tian, 2005; Tang and Riley, 2015).

Most ecosystem models partition soil organic matter into different pools that are decomposed at pool specific rates. These models simulate C dynamics by describing biological and physical mechanisms of SOC decomposition using first order kinetics, which are known to be simplistic and unable to fully capture microbially driven SOC dynamics (Guenet et al., 2018; Lawrence et al., 2009). Currently available SOC models demonstrate limited ability to reproduce the spatial

variability in SOC stocks, resulting in a large spread of future projections (Todd-Brown et al., 2013). One reason for this large spread may be that microbial mechanisms are not explicitly considered in C models. However, when microbial processes and their interactions with the physicochemical soil environment are included in the models, they have improved SOC predictions (Lawrence et al., 2009; Wieder et al., 2015). Nonetheless, explicit representation of microbial processes is challenging, due to the scale at which these processes occur (Wieder et al., 2015). Therefore, the predictive power of C dynamics models could be enhanced by identifying microbial processes and controls on microbial C cycling that are relevant at large scales, e.g., at the regional scale, and these controls could be used for model implementation and parameterization (Wieder et al., 2013; Hararuk et al., 2015). An example of such a control is the spatial variability in the Q_{10} of SOC decomposition; considering this variability has led to increased total (40 %) and microbial (25 %) respiration compared to the invariant Q_{10} values used in conventional global soil respiration models (Zhou et al., 2009).

4 Objectives

The fate of soil organic carbon is one of the largest uncertainties in predicting future climate and terrestrial ecosystem functions (Craine et al., 2010). Soil microorganisms mediate the decomposition of SOC and climate change has the potential to accelerate microbial SOC decomposition, providing positive feedback to climate warming. Altered soil temperature and moisture may influence microbial communities' SOC decomposition potential by modifying their growth strategies and by altering their interactions with the immediate environment (Balser and Wixon, 2009; Wallenstein et al., 2011; Hararuk et al., 2015). However, the intensity of SOC decomposition feedback to climate warming could vary between regions since both the effects of climate change drivers may differ between regions, and the decomposition rates of SOC may also vary due to regional differences. These include physicochemical and biological soil properties as well as land-use patterns that result in SOC quality differences (IPCC, 2014; Tifafi et al., 2018). Despite the critical role soil microorganisms play in controlling terrestrial C cycling and its feedbacks to climate change, there is limited understanding of the influence of physicochemical factors controlling microbial abundance and function. Therefore, the aim of this thesis was to understand the response of microbial SOC decomposition dynamics to changing soil resources and climate change factors, soil temperature and moisture, in regions differing in climatic and edaphic conditions. The hypothesis was that soil microbial communities and their respective extracellular enzymes would be adapted to climatic and edaphic conditions specific to each region and would, therefore, exhibit distinct temperature and moisture sensitivities. The aim of this thesis was addressed: 1) by clarifying the temporal patterns of degradation of SOC pools of varying quality (labile to recalcitrant), 2) by measuring abundance and respiration temperature sensitivities of functionally diverse microbial communities, and 3) by identifying controls on microbial SOC decomposition and its temperature sensitivity at different spatial scales.

Temporal patterns of degradation of SOC pools of varying quality were investigated using microbially produced extracellular enzymes as proxies. The aim was to investigate whether temperature and moisture sensitivities of extracellular enzyme activities vary at the seasonal scale and to test the validity of the "C quality temperature" hypothesis; i.e., to test whether or not labile C pools are less sensitive to temperature than recalcitrant C pools. Temperature and moisture sensitivities of three C cycling enzymes (β-glucosidase, xylanase, and phenoloxidase) which target

organic C pools of varying complexities (labile to rather recalcitrant and complex) were measured over a period of thirteen months, in two geographic areas differing in climatic and edaphic conditions, and two land-use types differing in SOC quality. Using a combination of laboratory measurements, field data, and mathematical tools, estimations of *in situ* enzyme potentials were made to clarify the temporal variations in degradation of C pools of different complexities. Furthermore, in view of extracellular enzymes as rate-limiting agents of SOC decomposition (Koch et al., 2007), it was tested whether temperature-based *in situ* enzyme potentials could explain measured soil respiration fluxes and whether adding moisture as an additional control variable improved the observed variations in soil respiration fluxes. The power of enzyme-based models in explaining soil respiration fluxes was also tested against the traditional temperature *vs.* soil respiration modelling approach.

Microbial communities are functionally diverse and decompose different fractions of organic C; thus, understanding the growth and respiration responses of microbial key players to temperature variation is essential for accurate prediction of climate-induced changes in SOC dynamics (Whitaker et al., 2014; Malcolm et al., 2008). Therefore, using soils from two areas and land-uses (see above), a short-term (36 day) microcosm experiment was established in the lab to investigate temperature sensitivities of soil bacteria and fungi. Three organic substrates of different qualities (cellobiose, xylan and coniferyl alcohol) were added to soil samples incubated at three temperature treatments (5, 15, and 25 °C) to mimic complex environments. The hypothesis was that the growth response of a soil microbial community depends strongly on environmental conditions (interactive temperature-substrate quality effects) that may alter inter- and intra-specific microbial community competition. Microbial community composition was determined by estimating phospholipid fatty acids (PLFA) and ergosterol content of the soil. Bacterial and fungal abundances were evaluated using the 16S rRNA gene and ITS fragment, respectively. Additionally, temperature-sensitive abundances of different bacterial taxa representing copiotrophs and oligotrophs were quantified using taxon-specific 16S rRNA quantitative PCR assays.

Identifying controls on microbial SOC decomposition and its temperature sensitivity at different spatial scales might help to improve the predictive power of C dynamics models. Seasonal variations in temperature sensitivity of soil extracellular enzymes and group-specific temperature responses of microbial key players to substrate of varying qualities investigated at the plot scale of

two different areas (see above) led to a third study, the focus of which was twofold; to investigate whether the temperature sensitivity of microbial SOC decomposition and related enzyme activities also vary at a spatial scale larger than the previously investigated plot scale, and to investigate the scale-specificity of factors controlling the temperature sensitivity of SOC dynamics. Specific biotic, physical, and chemical soil properties were tested as factors controlling basal soil respiration, its temperature sensitivity and the temperature sensitivity of two enzymes (β-glucosidase and xylanase) at landscape and regional scales. Output of the studies carried out in the framework of this thesis may have the potential to further parameterize and / or validate C dynamics models at the regional scale.

5 Modelling *in situ* activities of enzymes as a tool to explain seasonal variation of soil respiration from agro-ecosystems

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Abstract

Understanding *in situ* enzyme activities could help clarify the fate of soil organic carbon (SOC), one of the largest uncertainties in predicting future climate. Here, we explored the role of soil temperature and moisture on SOM decomposition by using, for the first time, modelled *in situ* enzyme activities as a proxy to explain seasonal variation in soil respiration. We measured temperature sensitivities (Q_{10}) of three enzymes (β-glucosidase, xylanase and phenoloxidase) and moisture sensitivity of β-glucosidase from agricultural soils in southwest Germany. Significant seasonal variation was found in potential activities of β-glucosidase, xylanase and phenoloxidase and in Q_{10} for β-glucosidase and phenoloxidase activities but not for xylanase. We measured moisture sensitivity of β-glucosidase activity at four moisture levels (12%–32%), and fitted a saturation function reflecting increasing substrate limitation due to limited substrate diffusion at low water contents. The moisture response function of β-glucosidase activity remained stable throughout the year. Sensitivity of enzymes to temperature and moisture remains one of the greatest uncertainties in C models. We therefore used the response functions to model temperature-based and temperature and moisture-based *in situ* enzyme activities to characterize seasonal variation in SOC decomposition. We found temperature to be the main factor controlling *in situ* enzyme activities. To prove the relevance of our modelling approach, we compared the modelled *in situ* enzyme activities with soil respiration data measured weekly. Temperature-based *in situ* enzyme activities explained seasonal variability in soil respiration well, with model efficiencies between 0.35 and 0.78. Fitting an exponential response function to *in situ* soil temperature explained soil respiration to a lesser extent than our enzyme-based approach. Adding soil moisture as a co-factor improved model efficiencies only partly. Our results demonstrate the potential of this new approach to explain seasonal variation of enzyme related processes.

5.1 Introduction

Soil carbon (C) stock is estimated to be > 1500 Pg C, significantly higher than atmospheric stock ~ 750 Pg C (Kirschbaum, 2000; Davidson and Janssens, 2006; IPCC, 2007). SOC, the largest pool in terrestrial C cycling (Kandeler et al., 2005), has the potential to act as a source or sink of greenhouse gases due to its dynamic interactions with the atmosphere (Lal, 2004). A large fraction of C is introduced into the atmosphere as CO_2 through microbial decomposition of organic matter (Frey et al., 2013). Temperature sensitivity of soil organic matter (SOM) decomposition has been given great attention (Davidson et al., 2012) due to the inherent relevance of kinetic theory (Davidson and Janssens, 2006). Expected warming of the earth's climate between 3 and 5°C over the next century (Bergfur and Friberg, 2012) may accelerate decomposition of SOC (Bengtson and Bengtsson, 2007) through faster processing of SOC by soil biotic communities and, therefore, affect the C source or sink functions of soils. The higher sensitivity of SOM decomposition, and in turn soil respiration, to temperature as compared to net photosynthesis makes investigations into the temperature sensitivity of C mineralization very important (Koch et al., 2007; Kirschbaum, 2000).

The temperature response of SOM decomposition depends upon its molecular structure; recalcitrant compounds have higher activation energies (E_a) than labile and, therefore, theoretically higher temperature sensitivity (Davidson and Janssens, 2006). Yet most existing C models consider a uniform temperature sensitivity of decomposition for organic matter pools of different stabilities (Fierer et al., 2005; Todd-Brown et al., 2012). This issue still needs to be resolved due to variations in findings related to the temperature response of different C pools (Zimmermann and Bird, 2012).

Extracellular enzymes (EE), produced by soil microorganisms, perform the rate-limiting step in SOM decomposition as well as nutrient cycling (Allison and Vitousek, 2005; Sinsabaugh, 1994). Most C models do not take extracellular enzyme kinetics explicitly into consideration (Allison et al., 2010). Recently efforts have been made to develop mechanistic models to simulate the combined effect of temperature, moisture and soluble-substrate supply on soil respiration by considering enzyme kinetics (Davidson et al., 2012). As almost half of the CO_2 released from soil is linked to decomposition of SOM by microorganisms and a large fraction of this respired CO_2

depends upon EE activity (Frey et al., 2013; Ryan and Law, 2005), adding enzyme kinetics to C models has the potential to improve climate change predictions (Allison et al., 2010).

Environmental factors, such as soil temperature, pH, diffusion constraints, and substrate availability and complexity modify microbial production, expression and temperature sensitivity of EE (Burns et al., 2013; Koch et al., 2007). For example, by analysing samples collected over different seasons from a forest soil Baldrian et al. (2013) found that seasonal variations in soil temperature strongly influenced SOM decomposition by changing the pool size and activity of EE. Different studies have focused on seasonal variations in the temperature sensitivities of soil enzymes (Koch et al., 2007; Brzostek and Finzi, 2012). However, it is still unclear which factors drive these seasonal trends (Jing et al., 2014). The complex interactions between enzymes and their environment and high variability of their temperature sensitivities makes it impossible to extrapolate single measurements across different temporal scales (Weedon et al., 2011). The current laboratory assays for measuring EE activities are performed under controlled conditions, which do not represent these complex interactions *in situ* (Henry, 2012). Moreover, this approach neglects the fundamental role of different factors, e.g. temperature and enzyme/substrate diffusion, in controlling *in situ* enzyme activities (Weedon et al., 2011). To illustrate the interactions of enzyme pool size and seasonal temperature sensitivity patterns in controlling *in situ* enzyme activities, Wallenstein et al. (2009) developed a predictive model of *in situ* β-glucosidase activities based on enzyme activities measured at different sampling dates, Q_{10} and daily soil temperature data from an arctic tundra site.

Little information is available on the effects of soil moisture on the temperature sensitivity of organic matter decomposition (Craine and Gelderman, 2011; Steinweg et al., 2012). Limiting soil moisture can cause a decline in diffusion rates of substrates and, therefore, in EE activity (Davidson and Janssens, 2006). As a consequence, increasing temperatures may not result in a positive feedback to climate change when soil moisture is a limiting factor (Allison and Treseder, 2008). Standard enzyme assays are performed in soil slurry (Poll et al., 2006; Kramer et al., 2013) for estimating enzyme potentials at non-limiting conditions neglecting diffusion constraint. Recently, Steinweg et al. (2012) developed an assay based on the use of fluorogenic substrates, to account for diffusion limitation at low water content and for non-homogeneous distribution of substrate in soil.

Previous studies have predicted the response of EE activity to *in situ* temperature and moisture (Steinweg et al., 2012; Wallenstein et al., 2009) and have yielded valuable insights into soil carbon dynamics. To date, however, the next step, that of using modelled *in situ* enzyme potentials as an explanatory tool for the seasonal variation of CO_2 respiration, is missing.

The goal of the present study is to explore the role of abiotic controls, i.e. soil temperature and moisture, on SOM decomposition by using modelled *in situ* enzyme activities as a proxy. We modelled *in situ* temperature-based potentials of three different enzymes (β-glucosidase, xylanase and phenoloxidase) targeting organic matter pools of different complexity, at two different study sites, with and without the presence of vegetation (fallow and vegetation plots). The selection of these three enzymes was based on the assumption that the targeted organic matter pools are representative for most of the soil organic matter pools. We also modelled *in situ* moisture-based β-glucosidase potential for both study sites and combined both temperature and moisture functions to illustrate the combined effect of both abiotic factors on enzyme potentials. To identify the similarities in seasonal patterns of modelled *in situ* enzyme activities with soil respiration and to prove the relevance of the modelling approach, we compared the modelled *in situ* enzyme activities with weekly measured soil surface CO_2–C fluxes. We hypothesized that (1) temperature and moisture sensitivity of enzymes targeting organic matter pools of different stability will change during the year. Furthermore, we expected that (2) measured soil CO_2 flux correlates strongly with the modelled *in situ* enzyme potentials, and we expected even stronger correlations with combined controls of soil temperature and moisture on *in situ* enzyme potentials.

5.2 Material and methods

Study site description

We investigated two study regions, with different climatic and edaphic conditions, which are part of the integrated research project "Agricultural landscapes under global climate change – processes and feedbacks on regional scale" (https://klimawandel.uni-hohenheim.de/). The first study site (48°31'7" N, 9°46'2" E) is located close to the city of Nellingen in the Swabian Alb region, while the second study site (48°55'7" N, 8°42'2" E) is located close to the city of Pforzheim in the Kraichgau region. The Swabian Alb (800–850 m a.s.l.) is characterized as an extensively used grassland and croplands region with cool and humid climate (mean annual temperature and precipitation ≤ 7°C and 800–1,000 mm, respectively, see Fig. S5.1a.). The Kraichgau region (100–400 m a.s.l.) is characterized by a warmer and drier climate (mean annual temperature and precipitation > 9°C and 720–830 mm, respectively, see Fig. S5.1b.) and intensive agriculture. Swabian Alb is a karst plateau of Jurassic limestone with soils classified as Calcic Luvisol, Anthrosol and Rendzic Leptosol whereas the Kraichgau region has loess parent material with soil developed into Stagnic Cambisol (WRB, 2007).

Experimental setup and soil sampling

To capture a range of soil carbon storage conditions, to investigate soils differing in organic matter input but which are typical for each region and to isolate more recalcitrant organic matter, three fallow plots (5 × 5 m) were installed in three selected agricultural fields in each study region in 2009. These plots were left fallow since 2009 until the time of sampling. Adjacent to fallow plots, vegetation plots (5 × 5 m) were selected in 2012 (vegetation type and period are given in Table S5.1). Each fallow and vegetation plot was considered as an individual block for statistical analysis. The fallow plots were managed by manual weeding and periodic spraying of glyphosate (Monsanto Agrar, GmbH, Germany) throughout the year. *In situ* soil temperature and moisture data of fallow plots were recorded by vertical installation of temperature (0 - 15 cm, 109 Thermistor Probe, Campbell Scientific, Ltd. Germany) and TDR probes (0 - 30 cm, CS625 Water Content Reflectometer, Campbell Scientific, Ltd. Germany) in each of the fallow plots. The same data for vegetation plots were obtained from temperature (at 2, 6 and 15 cm depths) and TDR probes

installed (at 5, 15 and 30 cm depths) close to eddy covariance (EC) stations in Kraichgau and Swabian Alb (3 EC stations per region).

Soil sampling (0-30 cm) was done each month from April 2012 to April 2013 and snow cover was removed in winter just before sampling. Four soil cores from each plot were thoroughly mixed and homogenized to obtain one representative sample. Samples were kept in cooling boxes and transferred to the laboratory. Soils were sieved (<2 mm) and water content was measured gravimetrically (60°C for 3 days). Soil samples were stored at -24°C until further analysis.

Soil-surface CO_2–C flux

Soil surface CO_2 flux was measured with two portable non-dispersive infrared field Environmental Gas Monitor instruments equipped with soil respiration chambers (10 cm diameter, 1171 cm^3 volume, EGM-2 and EGM-4 models, PP Systems Amesbury, Massachusetts, USA). Gas measurements were performed weekly, between 9:00 and 12:00 h, from 25.04.2012 to 05.04.2013. Three measurements were taken on each fallow and vegetation plot at each measurement date in both regions. On the vegetation plots, plant above ground biomass was excluded from chamber measurements but roots were still present which might have impaired CO_2 flux measurements. Flux measurements were not performed as intensively in winter as in summer due to high snow cover and difficulties in accessing the experimental fields.

Analyses

Microbial biomass carbon (C_{mic})

Microbial carbon was determined based on the chloroform fumigation-extraction method. For method specific steps, please see Kramer et al. (2012). Samples were extracted with 0.5 M K_2SO_4 (1:4 w/v).

Enzyme analyses

Temperature sensitivity assays

Temperature sensitivity assays of selected enzymes were performed on monthly collected soil samples starting from April 2012 till April 2013. Measurement of potential β-glucosidase activity was done according to Marx et al. (2001) with minor modifications. Substrate (4-methylumbelliferyl-β-D-glucopyranoside), standard (MUF) and MES-buffer were obtained from Sigma–Aldrich (St. Louis, USA.). Final concentrations of substrate and standard working solutions were 1 mM and 10 µM, respectively. Preliminary results showed that 1 mM substrate concentration was optimal for the investigated soils (data not shown). Briefly, 1 g soil (fresh weight from frozen samples) was suspended in 50 ml sterile water and particles disaggregated with an ultrasonic probe (50 J s^{-1} for 2 min). Fifty µl of soil suspension was added to each well of a microplate (PP microplate, black 96 well, Greiner Bio-one GmbH, Frickenhausen, Germany) together with 50 µl buffer and 100 µl of substrate solution. Each soil sample was replicated 8 times (8 wells in the microplate). Temperature sensitivity of β-glucosidase activity was measured at 5 temperatures; 6, 12, 18, 24, and 30°C. Soil plates were pre-incubated at their respective incubation temperatures for 30 min and one standard plate was prepared with standard concentrations of 0, 0.5, 1, 2.5, 4, and 6 µM. After pre-incubation, measurements were conducted at 0, 30, 60, 120 and 180 min with a fluorescence microplate reader (FLx800, BioTek Instruments Inc., Winooski, VT, USA) with excitation and emission filters at 360/460 nm, respectively. During incubation, plates were covered in order to minimize evaporation.

Xylanase activity was measured according to a modified method of Schinner and von Mersi, (1990). To measure the temperature sensitivity of xylanase activity, the assay was run at a range of incubation temperatures; 6, 17, 28, and 39°C, in addition to the optimum temperature of 50°C. Briefly, 5 g fresh soil was incubated in 50 ml falcon tubes with 15 ml substrate solution (1.2% xylan, from beech wood, suspended in 2 M acetate buffer solution at 45°C for > 1 h, at pH 5.5) and 15 ml acetate buffer solution (2 M, pH 5.5) for 24 h. Blanks were run with 5 g of soil suspended in 15 ml buffer solution only. After incubation, 15 ml substrate solution was added to blanks and all samples were centrifuged at 4422 × g for 30 min. An aliquot of 0.5 ml from each sample was used for the Prussian blue colour reaction. Two hundred µl of produced reaction-solution was pipetted

into a 96-well microplate (3 wells per sample) and absorption was measured photometrically at 690 nm using a microplate reader (ELx808, Absorbance Microplate Reader, BioTek Instruments Inc., Winooski, VT, USA).

Potential phenoloxidase (PO) activities were measured using tetramethylbenzidin (TMB) as a substrate (Johnsen and Jacobsen, 2008). Briefly, 0.4 g fresh soil was suspended in 50 ml of Na-acetate buffer solution (50 mM). Soils were disaggregated using an ultrasonic probe (50 J s^{-1} for 2 min). Two hundred µl soil suspension was added to each well of the microplate. Sample wells contained 50 µl substrate (12 mM) while blank wells contained the same quantity of buffer solution. Negative controls were run without soil suspension but with buffer and substrate solutions. Each sample was replicated three times. Plates were incubated at 25°C (additional temperature steps at 5, 10, 15, and 20°C for temperature sensitivity determination) and measurements were done after 0, 4, 8, 12 and 16 min of incubation on a microplate reader (ELx808, Absorbance Microplate Reader, Biotek Instruments Inc., Winooski, VT, USA) at 630 nm.

Moisture sensitivity assay

The moisture sensitivity assay was performed only with β-glucosidase, for fallow and vegetation plots, according to a modified method by Steinweg et al. (2012). We selected four sampling dates; April, August, and December 2012 and April 2013 for moisture sensitivity in both study regions.

Fresh soil samples were dried overnight at room temperature to reach a water content of ~ 2%. Following drying, soils were weighed in 50 ml falcon tubes according to 2 g dry matter and sterile distilled water was added dropwise to establish moisture treatments ranging from ~2, 10, 15, and 20% water content (w/w). Soils were mixed for 5–10 s with a spatula. Immediately after water addition, 250 µl of 2 mM substrate (4-methylumbelliferyl-β-D-glucopyranoside) was added followed by 5 s stirring. After substrate addition, tubes were incubated at 30°C for 8 min followed by the addition of 31 ml of 0.1 M MES buffer. Samples were vortexed for 5 s and centrifuged for 3 min at 344 × g. Two hundred µl supernatant were transferred to a 96-well microplate and fluorescence was measured as described above. Two measurements, 0 and 8 min after substrate addition, were made to follow fluorescence development. Standard curves were prepared for each individual sample and moisture level by adding varying amounts of distilled water and MUF standard to soils to reach final MUF concentrations of 0, 100, 300, and 500 µM. Preliminary tests

showed a dependence of the standard curve on soil moisture (data not shown) indicating variation in the extraction efficiency of the MUF standards. In addition, we used standard curves only after 8 min incubations as preliminary trials indicated no slope variation between 0 and 8 min. Addition of substrate and standard further increased soil moisture to ~ 12–32%, which spanned the range of soil moistures measured under field conditions in both study regions.

Data calculation and modelling approach

We calculated the temperature sensitivity of measured β-glucosidase and xylanase activities by calculating Q_{10} values. The following exponential function was used to illustrate the response of enzyme activity to temperature:

$$k(T) = k_0\, e^{aT} \quad (5.1).$$

where T represents the incubation temperature (°C), k_0 is the intercept with the y-axis at T = 0°C and a is the exponential coefficient. The exponential function was used because the measured response of enzyme activity in the lab was exponential within our selected temperature range. We used a to calculate the Q_{10} values, for each individual plot as follows:

$$Q_{10} = e^{10 \times a} \quad (5.2).$$

We modelled the temperature-based *in situ* enzyme potential of soils of both study regions based on the monthly determined exponential response function and Q_{10} values and the measured average daily soil temperature data. We used the exponential response function to calculate enzyme activities at 6°C rather than using the measured activities to minimize the influence of measurement uncertainty at low temperatures. In addition, linear interpolation of the exponential coefficient (i.e. a from Eq. (5.1)) produced different enzyme activities compared to linear interpolation of Q_{10}. Therefore, calculated enzyme activities at 6°C and Q_{10} values were used for linear interpolation between the sampling dates, in order to get enzyme activities corresponding to 6°C and Q_{10} values on a daily basis. This linear interpolation was necessary to calculate *in situ* enzyme activity for each day. We used the empirical model developed by Wallenstein et al. (2009) to model the temperature-based *in situ* enzyme activity as follows:

$$k(T) = R_6 Q_{10}^{\left(\frac{T-6}{10}\right)} \quad (5.3).$$

where R_6 is the calculated enzyme activity at 6°C and T is the *in situ* daily average soil temperature.

Moisture sensitivity of β-glucosidase activity was characterized by using a saturation function, where we assumed that substrate diffusion and, therefore, substrate availability for enzymatic reactions decreases with decreasing soil moisture. We used a nonlinear least-squares self-starting Michaelis–Menten saturation function (R Core Team, 2017) to fit a moisture response function to normalized enzyme activities using the following equation:

$$k(\theta) = \frac{V_{max}\,\theta}{K_m + \theta} \quad (5.4).$$

where V_{max} is the maximum normalized enzyme activity (no units) and K_m is the soil water content at which the enzyme activity reaches 50% of V_{max}. Θ is the soil water content. This saturation function holds true for enzyme data produced in our study since soil water contents, in laboratory assay as well as *in situ*, never went below 12%. For even lower soil water contents a concave upward function should be used with a clear threshold at the moisture level where hydraulic conductivity is lost (Hamamoto et al., 2010). In our study, normalized data on a per plot basis (normalization for individual fallow and vegetation plot in each month with respect to the maximum value) were used to account for specific pool sizes of β-glucosidase in individual plots which would have affected V_{max}, and, therefore, comparability between plots. This allowed us to pool normalized data of the three fallow or vegetated plots for each month and region to get a more reliable model fit based on a higher number of data points. For comparison, we pooled the data of the three fallow or vegetated plots for each month and region before normalization. Fitting Eq. (5.4) to normalized data provided a factor by which *in situ* enzyme activity is reduced when diffusion limitation occurs and allowed the combination with Eq. (5.5). We calculated the relative *in situ* β-glucosidase activity by taking average of V_{max} and K_m over the whole sampling period and also by linear interpolation of V_{max} and K_m between the sampling dates.

To estimate the combined effect of soil temperature and moisture on the *in situ* β-glucosidase activity, we applied a multiplicative approach:

$$k(T,\theta) = R_6 Q_{10}^{\left(\frac{T-6}{10}\right)} \times \frac{V_{max}\,\theta_{in\,situ}}{K_m + \theta_{in\,situ}} \quad (5.5).$$

To prove the relevance of our approach of modelling *in situ* enzyme potentials of soils and to see the similarities in seasonal patterns of modelled *in situ* enzyme activities and soil respiration, we compared the measured CO_2–C flux (g m^{-2} d^{-1}) with modelled *in situ* enzyme potentials over the whole sampling period. We used a modified Weibull function (Eq. (5.6)), to describe the relation between measured soil CO_2–C fluxes and modelled *in situ* β-glucosidase and xylanase activities (k_g), with a clear threshold at low *in situ* enzyme potential where no CO_2 efflux was recorded.

$$I(k_g) = f \times (1 - e^{-(\lambda k_g)^L}) \quad (5.6).$$

where λ and L are fit parameters and f is the maximum measured CO_2 flux (g CO_2–C m^{-2} d^{-1}).

Comparison between CO_2–C flux and *in situ* soil temperature was also done by fitting an exponential function (see Table 5.4). The accuracy of the fitted models was tested by calculating the model efficiency (EF) as described in Loague and Green (1991). Statistical analyses were performed in the R program for statistical computing and graphics (R Core Team, 2017). We used mixed-effect models fitted with maximum likelihood (Pinheiro et al., 2015) to test for the effects of season, treatment (fallow and vegetation) and region on microbial biomass, potential enzyme activities, Q_{10} values, and measured CO_2–C flux; block and plot were taken as nested random effects. In case of significant interactions, Tukey's HSD (Mendiburu, 2015) for comparison of treatments was used. CO_2–C flux data were square root transformed for mixed-effect model fitting to achieve normal data distribution and homogeneity of variances. The level of significance was set to $P < 0.05$ in all cases.

5.3 Results

C_{mic} and potential enzyme activities

Plant input increased microbial biomass C, on average, by 41% in Swabian Alb; in Kraichgau, up to 66% increase was recorded over the whole sampling period ($F_{1,4} = 1188.57$ $P < 0.01$, Fig. S5.2a, b). Statistical analysis revealed significant seasonal variation in C_{mic} in the Swabian Alb as well as in the Kraichgau region ($F_{12,96} = 6.51$ $P < 0.01$).

Potential β-glucosidase activity showed significant seasonal ($F_{12,96} = 7.21$ $P < 0.01$) as well as regional ($F_{1,4} = 26.76$ $P \leq 0.01$) dependence (Fig. 5.1a, b). In Kraichgau, β-glucosidase activity remained quite stable in the early summer, decreased in September and November and showed a comparative increase in winter, while soils in the Swabian Alb showed higher seasonal variation in β-glucosidase activities. Land use had significant effects on potential β-glucosidase activities in both regions ($F_{1,4} = 254.49$ $P < 0.01$); with 42% and 54% greater activities in vegetation plots than in fallow plots over the sampling period in Swabian Alb and Kraichgau, respectively.

Considering potential xylanase activity (Fig. 5.1c, d), we recorded significant differences between the fallow and vegetation plots ($F_{1,2} = 241.76$ $P < 0.01$) with 65% higher activity in vegetation plots over the sampling period, as well as a strong seasonal effect ($F_{12,47} = 6.81$ $P < 0.01$) in the Swabian Alb region. In the Kraichgau region, xylanase activity in fallow plots was sometimes below the detection limit and therefore no results are reported for these plots; however, a significant seasonal effect was recorded in the vegetation plots ($F_{12,24} = 2.87$ $P \leq 0.01$). No statistically significant differences were found between vegetation plots of either region; activities increased at the beginning of summer, declined between June and September, and remained stable during winter.

Potential phenoloxidase activity did not exhibit any significant effect of land use. Season had a significant effect on phenoloxidase activity ($F_{12,94} = 23.63$ $P < 0.01$) but this seasonal effect was similar in both regions (Fig. S5.3a, b). Phenoloxidase activity peaked in May, was at the lowest recorded range in June and showed a linear increase through the vegetation period. Highest activities were recorded in November, shortly after harvest. Phenoloxidase activity decreased during December and January and increased again in February, where higher soil temperatures were also recorded (Fig. 5.4c, d).

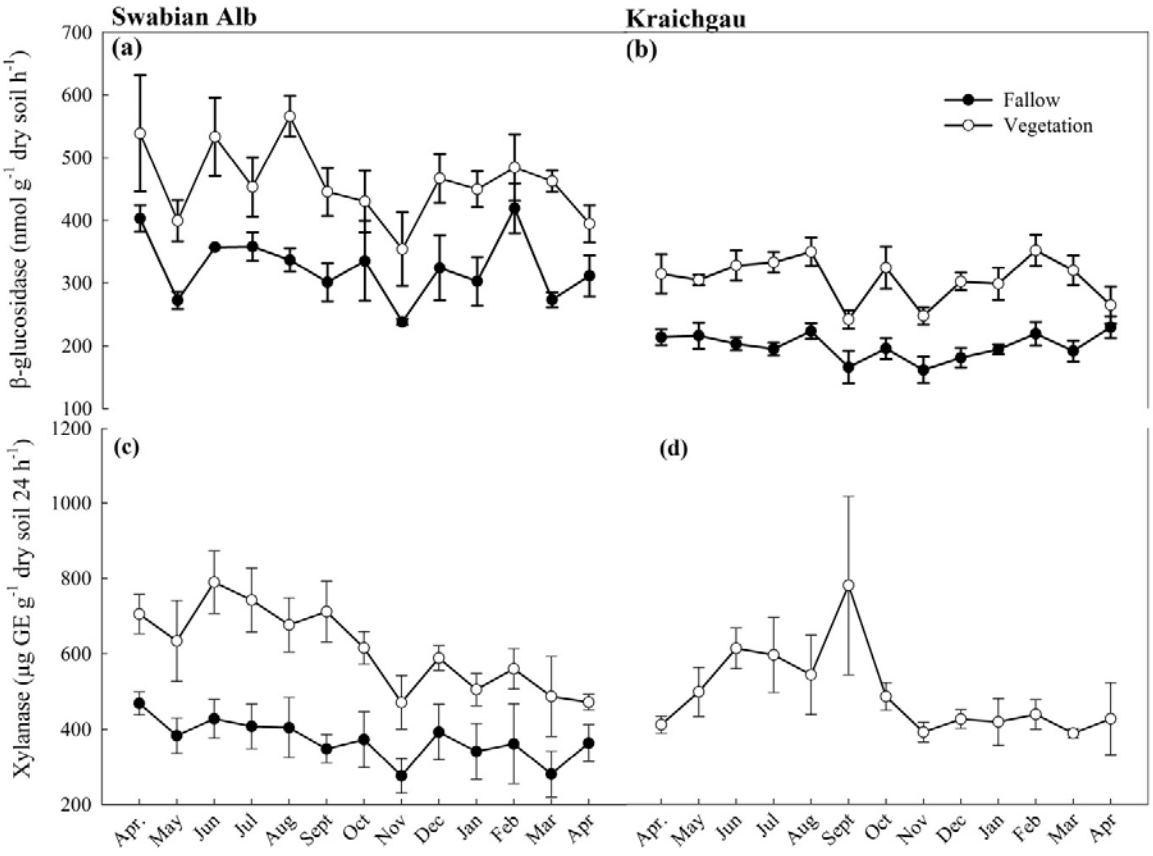

Fig. 5.1 Potential β-glucosidase activity measured at 30°C (a, b) and xylanase activity measured at 50°C (c, d) in both study regions. Bars represent standard error (n = 3). GE = glucose equivalents.

Table 5.1 Q_{10} values of β-glucosidase and xylanase measured at different laboratory incubation temperatures (starting from April 2012 till April 2013). Values represent average of field replicates (n = 3) and standard error is given in parenthesis. Q_{10} values for xylanase in Kraichgau fallow plots were not calculated due to assay limitations.

	β-glucosidase				**Xylanase**			
Month	Swabian Alb		Kraichgau		Swabian Alb		Kraichgau	
	Fallow	Vegetation	Fallow	Vegetation	Fallow	Vegetation		Vegetation
Apr	1.87 (0.01)	1.95 (0.09)	1.83 (0.09)	1.88 (0.06)	1.94 (0.07)	1.90 (0.04)		2.45 (0.08)
May	1.65 (0.05)	1.71 (0.04)	1.70 (0.06)	1.72 (0.04)	2.55 (0.37)	2.10 (0.19)		2.07 (0.09)
Jun	1.77 (0.04)	1.85 (0.06)	1.72 (0.03)	1.79 (0.05)	2.30 (0.31)	1.92 (0.06)		2.19 (0.21)
Jul	1.83 (0.02)	1.81 (0.04)	1.68 (0.05)	1.73 (0.06)	1.86 (0.17)	2.09 (0.20)		1.95 (0.09)
Aug	1.79 (0.02)	1.92 (0.03)	1.79 (0.02)	1.90 (0.05)	1.84 (0.30)	1.92 (0.29)		1.86 (0.41)
Sept	1.67 (0.03)	1.72 (0.01)	1.67 (0.01)	1.71 (0.06)	2.29 (0.31)	1.88 (0.14)		2.32 (0.38)
Oct	1.75 (0.03)	1.74 (0.03)	1.74 (0.02)	1.85 (0.004)	2.32 (0.25)	2.08 (0.20)		2.11 (0.18)
Nov	1.69 (0.02)	1.74 (0.01)	1.65 (0.02)	1.75 (0.05)	1.99 (0.42)	1.87 (0.05)		1.96 (0.28)
Dec	1.82 (0.06)	1.80 (0.03)	1.69 (0.05)	1.68 (0.08)	2.07 (0.24)	1.78 (0.03)		2.10 (0.02)
Jan	1.67 (0.04)	1.76 (0.01)	1.71 (0.03)	1.68 (0.04)	2.02 (0.16)	1.88 (0.12)		1.80 (0.14)
Feb	1.84 (0.06)	1.82 (0.05)	1.71 (0.04)	1.80 (0.04)	1.80 (0.13)	1.95 (0.12)		1.96 (0.14)
Mar	1.76 (0.04)	1.98 (0.08)	1.92 (0.03)	1.97 (0.02)	2.06 (0.29)	2.38 (0.07)		1.96 (0.07)
Apr	1.84 (0.02)	1.87 (0.04)	1.76 (0.02)	1.84 (0.01)	2.57 (0.14)	1.90 (0.13)		1.90 (0.41)

Temperature and moisture sensitivity of enzyme activity

By fitting an exponential function (Eq. (5.2)) to the measured enzyme activities at different incubation temperatures, Q_{10} values were calculated (Table 5.1, S5.2). Q_{10} of β-glucosidase activity showed a significant effect of sampling date ($F_{12,96} = 10.29$ $P < 0.01$) with values ranging from 1.6 to 2.1 in Swabian Alb and 1.5 to 2.0 in Kraichgau (Table S5.3). Xylanase showed comparatively higher Q_{10} values as well as higher differences than β-glucosidase with values ranging from 1.3 to 3.3 in Swabian Alb and 1.2 to 3.0 in Kraichgau (Table 5.1, S5.3). We did not detect any significant seasonal effects on the temperature sensitivity of xylanase activity. In both study regions, significant differences were found between the fallow and vegetation plots for Q_{10} of β-glucosidase ($F_{1,4} = 20.85$ $P = 0.01$), but not for xylanase. Phenoloxidase Q_{10} values ranged from 0.5 to 4.5 in Swabian Alb and 0.3 to 2.9 in Kraichgau (Table S5.3). In most of the samples, phenoloxidase did not show increased activity with increasing incubation temperatures. Temperature sensitivity of phenoloxidase activity differed between the regions ($F_{1,4} = 12.51$ $P \leq 0.05$) and also showed a seasonal effect ($F_{12,96} = 4.12$ $P < 0.01$).

We fitted an asymptotic function to explain the variation in β-glucosidase activity with changes in soil water content. β-glucosidase activity increased rapidly with increasing soil water content, with generally the lowest activity at 12% WC and the highest at 27 and 32% WC (Kraichgau: $F_{1,89}$ 42.25 $P < 0.01$, Swabian Alb: $F_{1,85}$ 65.58 $P < 0.01$), but were asymptotic once there was no longer a diffusion limitation for enzyme and/or substrate (Fig. 5.2a, b). On average, higher values of the fitted model parameters V_{max} and K_m were recorded in the fallow plots compared to vegetation plots in Swabian Alb region, while in the Kraichgau region an opposite trend was recorded (Table 5.2). V_{max} and K_m were not significantly different over the measurement dates in both study regions; therefore, averages for V_{max} and K_m across the year were used for further calculations. Model fitting was not possible for April 2013 data in the Swabian Alb. In August, in the fallow plots of Swabian Alb, much higher values of model parameters were recorded compared to other measurement dates. On average, fitted models showed higher model efficiencies in vegetation than in fallow plots. Moisture sensitivity data normalized on a per month basis also showed no significant differences of V_{max} and K_m in both study regions and model efficiencies were lower compared to data normalized on a per plot basis (data not shown).

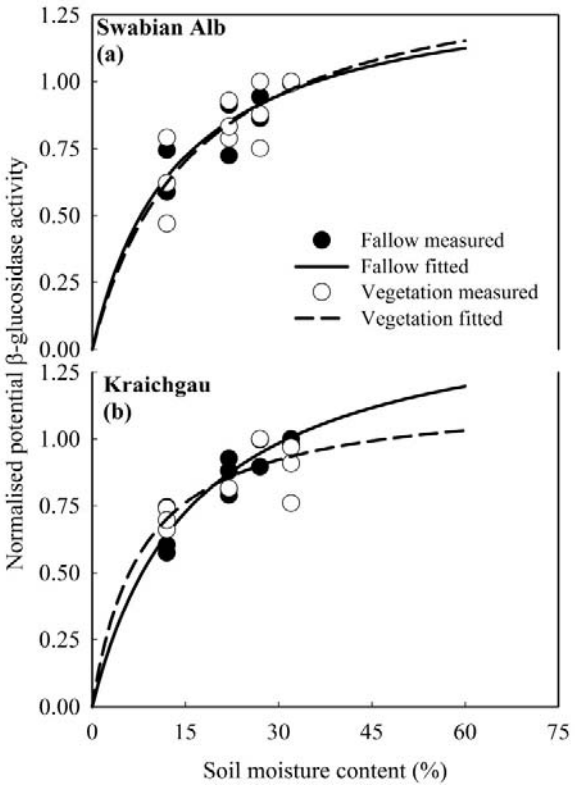

Fig. 5.2 Response of potential β-glucosidase activity to soil moisture for April 2012, in Swabian Alb (a) and Kraichgau (b). Data were normalized on per plot basis to fit the saturation function to laboratory-based enzyme activity measurements (n = 3 per moisture level). For Swabian Alb fallow plots, results are based on two field replicates.

Table 5.2 Model parameters extracted by fitting a saturation response function (Eq. (5.4)) to normalized enzyme activities and soil moisture (starting from April 2012 to April 2013). EF is the model efficiency with maximum value of 1, while n.d. represents "not determined".

	Swabian Alb						**Kraichgau**					
	Fallow			Vegetation			Fallow			Vegetation		
	V_{max}	K_m	EF	V_{max}	K_m	EF	V_{max}	K_m	EF	V_{max}	K_m	EF
April	1.38	13.7	0.8	1.5	16.3	0.7	1.5	16.5	0.9	1.2	8.04	0.6
August	38.7	1229	0.9	1.9	29.4	0.9	1.5	19.4	0.7	2.2	36.4	0.9
December	1.48	19.1	0.5	3.7	95.1	0.8	1.7	26.5	0.6	1.8	25.7	0.8
April	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.0	2.7	0.1	2.1	40.1	0.7

Modelled *in situ* enzyme potentials

We modelled *in situ* potential for β-glucosidase and xylanase. Since phenoloxidase activities showed an atypical temperature response in most soil samples (see above), we could not apply the modelling approach for this enzyme. In general, temperature-based *in situ* activities (Fig. 5.3a, b) were higher in the vegetation plots than fallow plots even though *in situ* soil temperatures were, on average, comparatively higher in fallow plots. Modelled *in situ* temperature-based β-glucosidase and xylanase activities increased during spring and throughout summer, started decreasing after September and remained stable during the winter. Activities increased again in April 2013 when soils began to warm.

We calculated the relative moisture-based enzyme potential of both study regions for β-glucosidase only (Fig. 5.3c, d). For Swabian Alb, we were able to fit the saturation function only for the year 2012. Predicted β-glucosidase activities were higher in the vegetation plots of Swabian Alb than in the fallow plots throughout the year. Low activities appeared during summer during periods of low moisture combined with high temperatures (Fig. 5.3c & 5.4c). Modelled β-glucosidase *in situ* moisture-based activities in Kraichgau were lowest in September (Fig. 5.3d). In both study regions, we recorded higher moisture-based β-glucosidase *in situ* activities during winter.

Adding moisture sensitivity as a co-factor together with temperature reduced the average activity, which was visible mainly in summer due to a reduced difference between summer and winter (Fig. 5.3e, f). Statistically significant differences were found for *in situ* β-glucosidase activities between temperature-based and moisture and temperature-based *in situ* activities (Table 5.3).

Table 5.3 Average modelled *in situ* β-glucosidase activities (nmol g^{-1} dry soil h^{-1}) for both study regions with standard error given in parenthesis. For Kraichgau, average of whole year data is presented (April 2012 till April 2013) while for Swabian Alb, average until December 2012 is given. Different letters within columns indicate significant differences of modelled activities between temperature-based (Eq. (5.3)) and combined (temperature + moisture, Eq. (5.5)) models for each treatment, at significance level $P < 0.01$.

	Swabian Alb		**Kraichgau**	
	Fallow	Vegetation	Fallow	Vegetation
Temperature-based, *k(T)*	143 (3.3) a	176.87 (3.5) a	75.3 (1.6) a	105.5 (2.2) a
Combined, *k(T,Θ)*	116.4 (2.4) b	160.5 (3.1) b	62.9 (1.2) b	85.8 (1.7) b

Table 5.4 Model efficiencies (EF) calculated by fitting modified Weibull function to weekly measured soil CO_2–C flux and modelled *in situ* enzyme potential for both study regions. Average CO_2–C flux for each land use (3 measurements × 3 plots) represented the respective data point against modelled *in situ* enzyme potential. Model efficiency for soil temperature and CO_2–C flux was calculated by fitting an exponential function given as follows: $CO_2 - C\ \text{flux} = a \times e^{b \times \text{temperature}}$ Maximum EF value = 1, n.d = not determined.

	Swabian Alb		**Kraichgau**	
	Fallow	Vegetation	Fallow	Vegetation
CO_2–C flux as a function of temperature-based in situ enzyme activity				
β-glucosidase	0.47	0.78	0.56	0.65
Xylanase	0.35	0.76	n.d	0.64
CO_2–C flux as a function of temperature & moisture-based in situ enzyme activity				
β-glucosidase	0.35	0.66	0.57	0.72
CO_2–C flux as a function of in situ soil temperature				
	0.42	0.63	0.38	0.59

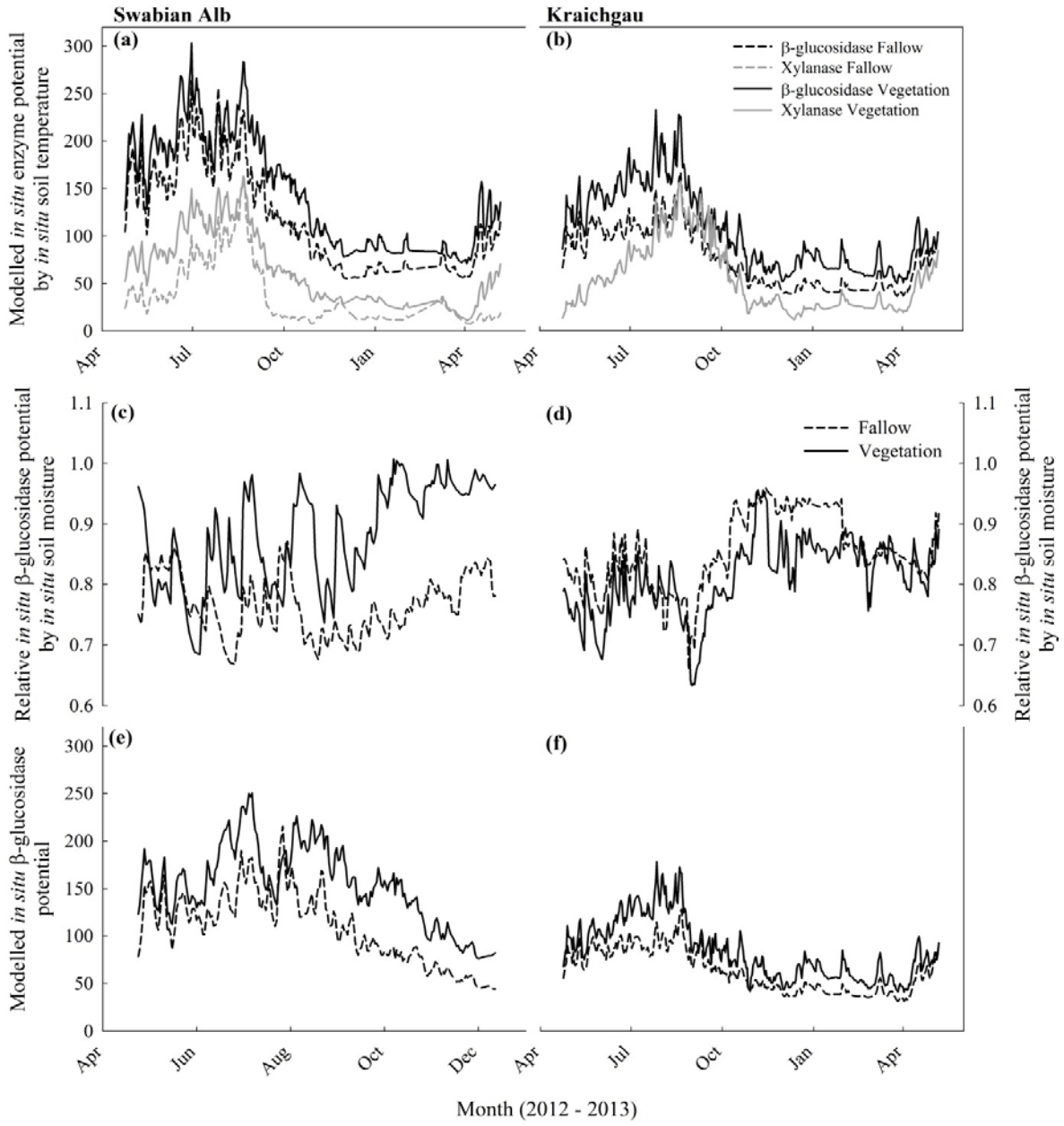

Fig. 5.3 Modelled *in situ* temperature-based β-glucosidase and xylanase activities in Swabian Alb (a) and Kraichgau (b) region. While (c) and (d) represent the relative *in situ* moisture-based β-glucosidase potential and (e) and (f) represent combined effect of soil temperature and moisture on *in situ* β-glucosidase activity in Swabian Alb and Kraichgau, respectively.

Soil respiration

Land use had a significant effect on the seasonal variability of soil respiration in both study regions ($F_{1,4}$ = 55.32 P < 0.01; Fig. 5.4a, b). In the vegetation plots, soil respiration increased during summer and peaked in September (Fig. 5.4a) or shortly after September (Fig. 5.4b). Respiration declined by mid-October, when soil temperature had also declined (Fig. 5.4c, d).

We tested the relevance of our modelling approach (prediction of *in situ* enzyme potential) by fitting asymptotic model to the predicted *in situ* enzyme activities and measured CO_2–C efflux. This model fit was chosen as measured CO_2–C efflux increased linearly in the beginning but reached an asymptote at higher predicted *in situ* enzyme activities (Fig. 5.5). Accuracy of the fitted model is presented in terms of EF (Table 5.4). The fitted model efficiencies for β-glucosidase and xylanase were higher in vegetation plots in comparison to fallow plots in both study regions when only *in situ* temperature was considered as a controlling factor. Adding soil moisture as a co-factor for controlling β-glucosidase activities improved model predictions only in the Kraichgau region (Table 5.4).

As seasonal soil CO_2 flux can also be explained, to some extent, by temporal variations in soil temperature (Davidson et al., 1998), we looked how well our enzyme-based model explains variation in soil respiration when compared to a simple exponential response function of soil respiration to *in situ* soil temperature (temperature-based model). In both regions, the enzyme-based model performed better than the temperature-based model when soil temperature was considered as the main controlling factor (Table 5.4).

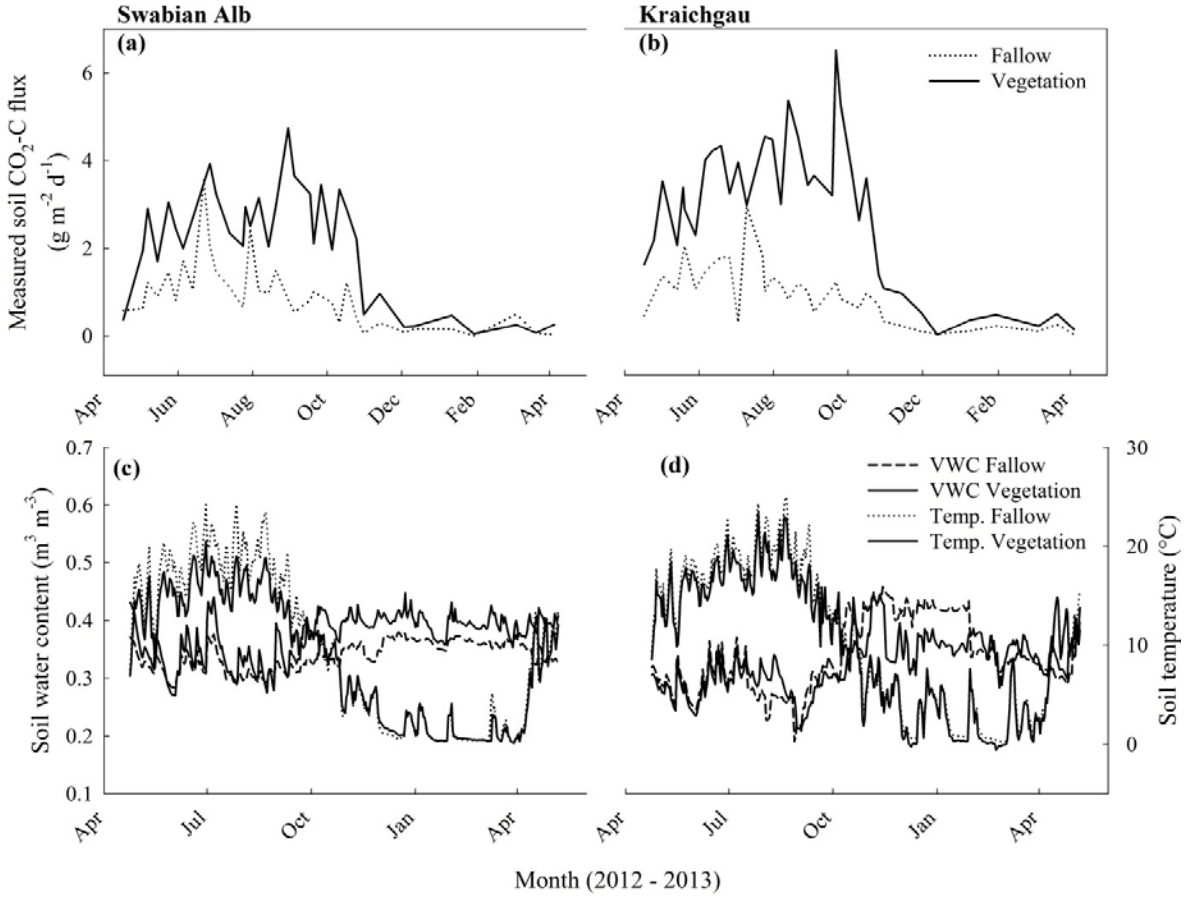

Fig. 5.4 Seasonal variation of weekly measured soil CO_2–C flux from April 2012 to April 2013 in Swabian Alb (a) and Kraichgau (b) in fallow and vegetation plots. While average daily *in situ* soil temperature (°C) and volumetric soil water content (VWC, m^3 m^{-3}) recorded over the whole sampling period in both fallow and vegetation plots of Swabian Alb and Kraichgau is shown in (c) and (d), respectively.

5.4 Discussion

Understanding temperature and moisture sensitivity of enzymatic reactions might play an important role in explaining seasonal variation of soil respiration. We found significant seasonal variation in temperature sensitivities of enzymes targeting different substrate pools, which could have important implications for the relative decomposition of SOM and modification of C cycling in predicted climate change scenarios. Seasonal variability in soil temperature was found to be the main controlling factor of the *in situ* enzyme activity. The present study is the first of its kind to explain temporal variation of soil respiration using *in situ* enzyme potential as a proxy where applied models successfully explained variation of enzyme related processes.

Seasonal variation in potential enzyme activities

Global climate change has the potential to affect C cycling by affecting the physiology of primary drivers of SOM decomposition such as microbial communities, which use mainly extracellular enzymes as proximate agents of SOM decomposition (Li et al., 2014). In our study, intensive soil sampling over the whole year from two regions differing in climatic conditions and land use (with the establishment of fallow plots) provided the opportunity to detect seasonal variation as well as land use effects on potential activities of enzymes targeting substrates of varying complexity. We observed significant seasonal variation in potential activities of the three investigated enzymes (Fig. 5.1, S5.3). Degree of variation across the year was similar for β-glucosidase and xylanase while phenoloxidase, in comparison to the other two enzymes, showed two and three to four fold higher variation in Swabian Alb and Kraichgau, respectively. Potential enzyme activity rates are controlled by enzyme pool size, which in turn may be affected by soil temperature and moisture causing seasonal variation in potential enzyme activities (Steinweg et al., 2013). Under stress conditions like drought, soil microbial communities change their strategy of resource allocation from growth and resource acquisition to survival by reducing enzyme production (Schimel et al., 2007). Therefore, seasonal changes in enzyme pool size may be explained either by reduced enzyme production by the same community (Schimel et al., 2007) or by reduced microbial community size (Hueso et al., 2011). However, substrate availability dynamics (Sinsabaugh, 2010) may also explain the observed significant differences in land use for β-glucosidase and xylanase activities, which occur due to differences in inputs from different plant communities, resource

allocation, as well as C input through roots and root exudates (Hargreaves and Hofmockel, 2014). The seasonal variation might also occur due to moisture limitation during periods of low rainfall or under frozen conditions, causing substrate limitation at the enzyme active site (Davidson and Janssens, 2006) and reducing the 'return on investment' in enzyme production (Schimel and Weintraub, 2003). Soil temperature variation could also explain variation in enzyme activities over the investigated time period (Fenner et al., 2005; Davidson and Janssens, 2006; Baldrian et al., 2013). In our study, considerably lower potential enzyme activities were recorded during winter when soil temperature was low. Baldrian et al. (2013) conducted a study in a forest ecosystem and also found low activities of different enzymes during winter in the O and Ah soil horizons. Authors concluded that soil temperature is the main factor controlling seasonal variation in enzyme activities. However, it is difficult to separate the confounding interactions of soil microbial community composition, soil microbial community response under stress conditions, soil temperature and moisture, plant growth changes, as well as organic matter input (Bell et al., 2010; Chen et al., 2004).

We tested for vegetation cover effects on the relation between measured enzyme activities in both study regions. β-glucosidase activity was not significantly correlated to xylanase and phenoloxidase activities in both treatments as well as both study regions (Fig. S5.4, S5.5). Similar results have been reported for the relation of β-glucosidase and phenoloxidase activities by Sinsabaugh and Shah (2011), who argued that a non-significant relation between these two enzyme classes shows variation in enzyme production, activity as well turnover at the community and ecosystem scale.

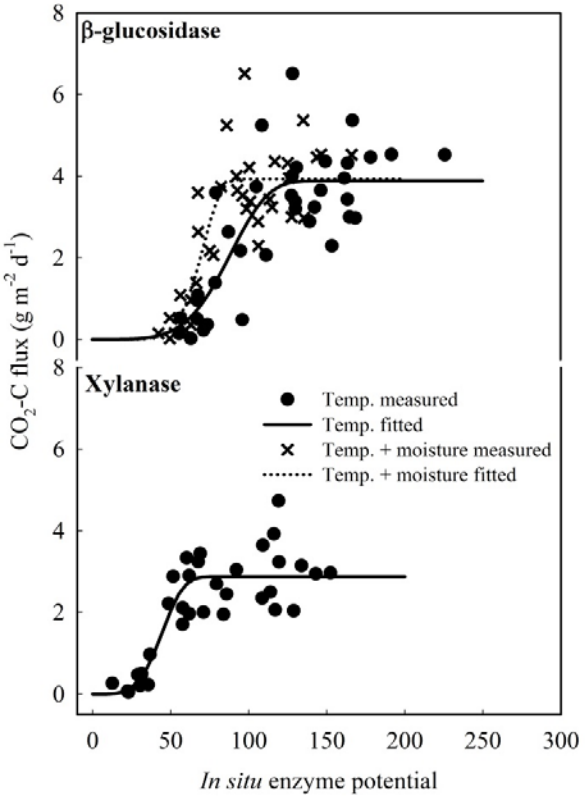

Fig. 5.5 Response of measured CO_2–C efflux to modelled *in situ* β-glucosidase potential as a function of soil temperature alone and in combination with soil moisture in vegetation plots of Kraichgau, and modelled *in situ* xylanase potential as a function of soil temperature alone, in vegetation plots of Swabian Alb.

Temperature and moisture sensitivity of enzyme activities

If there is no change in an enzyme pool, variations in soil temperature and moisture may have the potential to change soil respiration rate by affecting microbial functions (Gougoulias et al., 2014). In the present study, the cellulose degrading enzyme β-glucosidase showed lower average Q_{10} (1.78 in Swabian Alb, 1.76 in Kraichgau) than xylanase, the xylan degrading enzyme (2.04 for both regions). Whereas β-glucosidase targets oligosaccharides derived from the decomposition of cellulose (Cañizares et al., 2011), different xylanases are responsible for the degradation of complex hemicelluloses (Wyman et al., 2004). Therefore, the multi-enzyme system for the degradation of xylan might differ in its temperature sensitivity from that of β-glucosidase (Trasar-Cepeda et al., 2007). Differences in Q_{10} may also be explained by the difference in E_a of both enzymes. For example Wang et al. (2012) showed that enzymes targeting labile substrates had

lower E_a than enzymes targeting more recalcitrant substrates; therefore, this difference in E_a may cause the difference in temperature sensitivity (Davidson and Janssens, 2006).

Our hypothesis, that the temperature sensitivity of enzyme activity depends upon season, was partially true; we found seasonal variability in Q_{10} for β-glucosidase and phenoloxidase, but not for xylanase. This observed variability could be due to different isoenzymes, produced by the same or different microorganisms over the season, having different temperature responses (Wallenstein and Weintraub, 2008; Jing et al., 2014). Q_{10} values of β-glucosidase found in our study were within the range reported by Koch et al. (2007) and Wang et al. (2012). We found an increase in average Q_{10} values only in β-glucosidase and phenoloxidase in winter and early spring for both study regions, when there were also lower recorded soil temperatures. In agreement with our study, Kirschbaum (1995) also found that the temperature sensitivity of decomposition increases with decreasing temperature. This high sensitivity may be achieved by increased catalytic efficiency of enzymes through enhanced conformational flexibility under cold conditions (Georlette et al., 2004; Bradford et al., 2008).

Though we detected seasonal variability in the Q_{10} of phenoloxidase, we did not detect any clear pattern in phenoloxidase activity with increasing incubation temperatures in the lab assay. A possible reason for this could be large spatiotemporal variation (Sinsabaugh, 2010), which might be due to enzyme stabilization on mineral surfaces and inefficient interactions of the enzyme-substrate complex (A'Bear et al., 2014). Since phenoloxidase may not follow Michaelis–Menten kinetics, as do other enzymes, its assays are complex and difficult to optimize (German et al., 2012). We found an effect of region on the Q_{10} values of β-glucosidase and phenoloxidase. Although these two regions differ in soil organic matter content (Swabian Alb > Kraichgau), it is not easy to draw any conclusion as to which soil properties caused these variations and therefore, factors affecting the regional temperature sensitivity of enzyme activity need to be explored further.

Soil microbial interactions with their physical environment can also be affected through changes in soil moisture (Cook and Orchard, 2008), a factor having the potential to affect the C cycle and soil respiration in a global climate change context (Poll et al., 2013). In our study, potential β-glucosidase activities responded positively to short-term moisture manipulations, showing significant increases in activity when soil moisture increased from 12 to 27 or 32% WC (Fig. 5.2).

Similar results were found by Steinweg et al. (2012) where β-glucosidase activities generally increased with soil water content. According to Hinojosa et al. (2004) enzyme activities increase when diffusion limitation is alleviated as a response to increased soil moisture and improved access to either already available organic matter or to newly produced C from cell lyses due to drought stress. However, under dry soil conditions, enzymes adsorption to soil particles may cause changes in their conformation that might result in reduced substrate affinity and hence activity (Kandeler, 1990).

We fit an asymptotic model to increasing enzyme activity with increasing soil moisture because we assumed that at low soil moisture levels, substrate and enzyme diffusion is limited; therefore, enzymes face low substrate concentrations in their very direct environment, resulting in low activities. At high soil moisture, diffusion does not limit substrate availability and factors other than moisture (e.g. soil temperature, enzyme concentration) become more dominant. We used the average of fitted parameters (V_{max} and K_m) because, in contradiction to our hypothesis, we did not find any significant differences in these parameters over the selected sampling dates. One possible reason could be that we did not reach the lower limit of soil moisture during the laboratory assay. At very low soil moisture, seasonal variation in conformational adaptation of isoenzymes to drought may have been more obvious than at the investigated water content range of 12–32%. However, the investigated moisture range in our study covered the observed range of soil moisture in both regions. Another possible reason for seasonal non-significance is the observed high variation in overall enzyme activity as well as in model parameters, which could be reduced if a larger range of soil moistures were used than in our study. Other factors controlling seasonal non-significance of model parameters might be soil texture and pore distribution. These two factors control substrate diffusion, which is a limiting factor for its decomposition at low moisture (Manzoni et al., 2012a; Mtambanengwe et al., 2004). Soil texture does not change over the season; therefore, one might expect no variation in moisture sensitivity across the season. Nevertheless, using the investigated range of soil moistures, the models fitted the data well and produced good model efficiencies (Table 5.2).

Modelled *in situ* enzyme potentials

We modelled *in situ* activities of enzymes targeting different carbon pools (β-glucosidase and xylanase) using their temperature and/or moisture sensitivity and measured *in situ* soil temperature and moisture. Since currently it is not possible to measure *in situ* enzyme activity directly in the field, only a few studies have modelled *in situ* enzyme activities from different soils and climatic conditions, by using a combination of enzyme activities in the lab and field measurements of soil temperature and moisture (Steinweg et al., 2012; Bárta et al., 2014).

When considering temperature as the only controlling factor, *in situ* activities of β-glucosidase and xylanase increased in spring through summer and declined in autumn through winter in both study regions (Fig. 5.3a, b). Temperature-based modelled *in situ* enzyme activities followed the seasonal variation in soil temperature in our study, as soil temperature variation, rather than seasonal changes in enzyme pool size, is the main factor controlling *in situ* enzyme activities (Baldrian et al., 2013). Reduced *in situ* enzyme activities observed during winter could likely be explained by substrate availability, e.g. reduced C input from above ground biomass, whereas enzyme production is limited by available C and N (Brzostek and Finzi, 2011). Therefore, microorganisms produce fewer enzymes resulting in lower *in situ* enzyme potential. On the other hand, at higher temperatures, e.g. during the growing season, more substrate is available for microbial decomposition. High substrate availability is also achieved by destabilizing SOM-mineral matrices at high temperature (Conant et al., 2011) causing a considerable increase in observed enzyme potential. Temperature increase also increases substrate diffusion rate, which leads to efficient substrate catabolism by enzymes (Brzostek and Finzi, 2012) and, therefore, a net increase in enzyme activities.

Diffusion also controls substrate transport through soil water films to enzyme reactive sites (He et al., 2014), which may also affect *in situ* enzyme potential. We modelled *in situ* moisture sensitivity of β-glucosidase activity to explain how diffusion limitation affects substrate availability, diffusion of enzymes, or enzyme catalysed-reaction products in the field. Moisture-based *in situ* β-glucosidase activity showed, in general, a steady increase from summer through winter in Swabian Alb and Kraichgau following patterns of measured *in situ* soil moisture. Upon drying, soil water-filled pores are reduced, resulting in limited microbial activity due to their limited motility and substrate availability (Manzoni et al., 2012a). Under these circumstances, the cost of enzyme

production is greater than the benefit from their activity in terms of nutrient availability (Allison and Vitousek, 2005), causing a decrease in net activity.

In our study, soil temperature explained > 90% variation in *in situ* β-glucosidase activity when temperature was considered as the only control of *in situ* enzyme activity. Adding soil moisture as a co-factor controlling *in situ* enzyme activity significantly decreased average *in situ* β-glucosidase activity (Table 5.3). Diffusion limitation due to periods of low moisture content might have overcome the positive effect of temperature on enzyme activity during summer. This trend was also observed in the study by Steinweg et al. (2012), where lack of soil moisture resulted in a strong negative effect on temperature and moisture-based *in situ* β-glucosidase activity.

Explaining soil respiration using *in situ* enzyme potential

Measured soil respiration in our study represents heterotrophic and root respiration except in the fallow plots where it solely represents heterotrophic flux from soil. We found significant seasonal variation in soil respiration that can be explained by changes in soil temperature and moisture, affecting ecosystem productivity and the rate of SOM decomposition (Han et al., 2007). Other factors, for example changes in substrate quantity, quality, and availability, might also explain seasonal variation in soil respiration (Buchmann, 2000).

We fitted an asymptotic model to explain the relation between measured CO_2 efflux and *in situ* enzyme potential because the asymptotic model was superior to a linear relationship. Because enzymatic products must overcome diffusion, microbial uptake, and metabolic processing before CO_2 is produced, a linear relation between *in situ* enzyme activities and soil respiration is therefore highly unlikely.

Another possible factor influencing a non-linear response could be reduction in soil microbial activities due to reduced substrate availability (German et al., 2011) through dynamic input of organic matter via roots, root exudates and leaf litter over the season (Hargreaves and Hofmockel, 2014). Wan and Luo (2003) showed a significant reduction in soil respiration with reduced substrate supply. Moreover, substrate limitation for microorganisms / enzymes could also result from substrate spatial variation in the soil (Resat et al., 2012). Microbial utilization of substrate depends upon, along with other factors, the position of the substrate within the soil matrix

(Kandeler et al., 2005). Physical protection of substrate by soil aggregates or adsorption onto mineral surfaces (Davidson and Janssens, 2006) may hinder its decomposition by microbial communities. Therefore, at high enzyme potential, substrate in the very direct environment of the enzyme might be limiting and this high enzyme potential therefore cannot be directly translated into CO_2 production.

High *in situ* enzyme potential is related mainly to higher soil temperatures, which occur in summer when water may become limiting either due to soil warming (water evaporation and plant water consumption), or less precipitation, which was also observed in our study. Soil microorganisms acclimate to dry conditions by changing their resource use strategy, spending more on survival than growth, e.g. through production of osmolytes (Schimel et al., 2007). Stress conditions may also force a microbial community shift towards those having less mass-specific respiration (Schimel et al., 2007; Billings and Ballantyne, 2013). This microbial community shift and physiological adaptation might also explain the saturation of respired CO_2 at high *in situ* enzyme potential observed in our study. Since we did not measure microbial community composition in this study, we can only speculate about microbial community shifts and observed CO_2 responses at *in situ* enzyme potential. This aspect still needs to be explored.

The non-linearity between CO_2 release in the field and *in situ* enzyme potential could also be explained by differences in microbial carbon use efficiency, which is affected both by differences in substrate availability and quality and by different nutrient availabilities, e.g. N, P, during the season (Manzoni et al., 2012b; Ågren et al., 2001; Frey et al., 2013). However, enzyme production could also be driven by availability of these nutrients in the soil (Allison and Vitousek, 2005). In addition, EE involved in different key processes of C-, and N-cycling might have different responses to temperature and/or moisture (Koch et al., 2007; Bárta et al., 2014). Therefore, differences in sensitivity might result in a decoupling of substrate release by β-glucosidase activity and further processing during CO_2 production.

In both regions of our study, abiotic factors exerted different controls on soil respiration using *in situ* potential of different enzymes as a proxy. Our results indicate that the Weibull function fitted the data well since this assumed a certain threshold of enzyme activity, ~ 50 nmol g^{-1} dry soil h^{-1} and ~ 12 µg GE g^{-1} dry soil $24h^{-1}$ for β-glucosidase and xylanase, respectively, below which

detection of the respired CO_2 may not be possible. Since EE retain their catalytic ability under cold conditions (Bremner and Zantua, 1975; Blankinship et al., 2014) and enzymatic products are being formed, further processing of these products to CO_2 production may decline, resulting in a lag phase such as that observed in our study for respired CO_2 at lower *in situ* enzyme potential (Fig. 5.5). Possible factors involved in this decoupling could be cost-intensive physiological acclimations adapted for survival by soil microorganisms, e.g. changes in membrane lipids and production of shielding and anti-freezing proteins (Schimel et al., 2007) reducing microbial growth, or higher sensitivity of microbial intracellular metabolism e.g. as a result of limited liquid water (Mikan et al., 2002), causing changes in microbial metabolic activity under freezing conditions. Upon soil thawing, a peak of CO_2 efflux is often observed (Schimel and Clein, 1996), generally associated with metabolism of easily decomposable organic matter originating from cell lyses (Feng et al., 2007). This peak may also be due to metabolism of extracellular enzyme-catalysed products produced during freezing conditions, but this aspect needs to be explored further.

Our modelled *in situ* temperature-based enzyme activities, as initially expected, successfully explained seasonal soil respiration efflux with model efficiencies ranging from 0.47 to 0.78 for β-glucosidase, and 0.35 to 0.76 for xylanase, proving the relevance of our modelling approach. Therefore, our results indicate that enzymes that degrade labile C compounds represent *in situ* CO_2 production better than enzymes that degrade more complex substrates (Table 5.4). The labile C pool contributes up to 90% of the total respired CO_2 efflux from soil to the atmosphere (Wan and Luo, 2003), probably due to higher decomposition at high temperatures (Mikan et al., 2002). However, for long term stabilization of SOC, decomposability of the stable pool is more important due to the higher temperature sensitivity of decomposition of this pool (Frey et al., 2013), which can result in a positive feedback to atmospheric CO_2.

Adding soil moisture as a co-factor in modelling *in situ* enzyme potential improved CO_2 prediction in the Kraichgau but not in the Swabian Alb region, partly supporting our hypothesis. This could be explained by overall differences in soil moisture in both regions (comparatively lower soil moisture recorded in the Kraichgau than in the Swabian Alb, particularly during the growing season Fig. 5.4c, d), and also due to short periods with limited soil moisture content. Therefore, in the Kraichgau region, due to a strong moisture effect together with temperature, better model efficiencies were obtained. Adding soil moisture as a co-factor increased soil respiration for a given

in situ enzyme potential (Fig. 5.5). This counterintuitive effect suggests that calculating temperature-based *in situ* enzyme potentials underestimates catalytic rates of soil enzymes, which underlines the above mentioned substrate limitation of EE.

We tested our new approach of enzyme-based variation on seasonal soil CO_2 efflux with a conventional approach of soil temperature-based variation of CO_2 efflux (Table 5.4). Obtained model efficiencies indicate that our enzyme-based model explained variation in CO_2 efflux better than the soil temperature-based model in both study regions. Though it is not clear if changes in enzyme catalytic rates will change soil respiration due to the uncertainty of dominant controlling factors (Billings and Ballantyne, 2013), our results exhibit the potential of our new approach to explain temporal variability in enzyme related processes.

In conclusion, this is, to our knowledge, the first study considering temperature and moisture-based *in situ* enzyme potentials to explain CO_2 efflux. Our study demonstrates that soil enzymes, as the proximate agents of SOM decomposition, respond differently to changes in soil temperature and moisture. Therefore, in the context of predicted climate change, soil enzymes have the potential to affect feedback mechanisms between agro-ecosystems and the atmosphere.

5.5 Acknowledgements

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6 Dynamics of soil respiration and microbial communities: interactive controls of temperature and substrate quality

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Abstract

Soil microbial communities mediate soil feedbacks to climate; a thorough understanding of their response to increasing temperatures is therefore central to predict climate-induced changes in carbon (C) fluxes. However, it is unclear how microbial communities will change in structure and function in response to temperature change and to the availability of organic C which varies in complexity. Here we present results from a laboratory incubation study in which soil microbial communities were exposed to different temperatures and organic C complexity. Soil samples were collected from two land-use types differing in climatic and edaphic conditions and located in two regions in southwest Germany. Soils amended with cellobiose (CB), xylan, or coniferyl alcohol (CA, lignin precursor) were incubated at 5, 15 or 25 °C. We found that temperature predominantly controlled microbial respiration rates. Increasing temperature stimulated cumulative respiration rates but decreased total microbial biomass (total phospholipid fatty acids, PLFAs) in all substrate amendments. Temperature increase affected fungal biomass more adversely than bacterial biomass and the temperature response of fungal biomass (fungal PLFAs, ergosterol and ITS fragment) depended upon substrate quality. With the addition of CB, temperature response of fungal biomass did not differ from un-amended control soils, whereas addition of xylan and CA shifted the fungal temperature optima from 5 °C to 15 °C. These results provide first evidence that fungi which decompose complex C substrates (CA and xylan) may have different life strategies and temperature optima than fungal communities which decompose labile C substrate (CB). Gram-positive and gram-negative bacteria differed strongly in their capacity to decompose CB under different temperature regimes: gram-positive bacteria had highest PLFA abundance at 5 °C, while gram-negative bacteria were most abundant at 25 °C. Bacterial community composition, as measured by 16S rRNA gene abundance, and PLFAs showed opposite temperature and substrate decomposition trends. Using multivariate statistics, we found a general association of microbial life strategies and key members of the microbial community: oligotrophic *Alphaproteobacteria* and *Acidobacteria* were associated with complex substrates and copiotrophic *Actinobacteria* with labile substrates. Our study provides evidence that the response of C cycling to warming will be mediated by shifts in the structure and function of soil microbial communities.

6.1 Introduction

Soil has long been a focus of climate change studies due to its large carbon (C) stock relative to plant biomass and atmospheric C (Karhu et al., 2014; Todd-Brown et al., 2014). Microbial soil organic carbon (SOC) decomposition is a vital process controlling C storage in terrestrial ecosystems and it is widely accepted that soil microorganisms have the potential to accelerate climate change by adding more C in the form of CO_2 to the atmosphere (Yuste et al., 2011; Thakur et al., 2015; Auffret et al., 2016). Different studies argue that climatic and environmental variables including soil texture, temperature, moisture, and C quantity and quality are the main controls on SOC decomposition (Davidson and Janssens, 2006; Cleveland et al., 2007; Karhu et al., 2014). However, it is unclear how these abiotic variables interact with biotic factors and alter microbial substrate utilization response under varying temperatures (Zogg et al., 1997; Waldrop and Firestone, 2004; Cleveland et al., 2007; Tang et al., 2018). Therefore, understanding how functionally diverse microbial communities alter their abundance and respiration response to temperature is essential if we are to predict climate-induced change in respiration in different ecosystems (Malcolm et al., 2008).

Microbial abundance, metabolic activity, substrate uptake rates, and community structure are strongly regulated by temperature variation (Stres et al., 2008; Conant et al., 2011; Bradford, 2013). However, little is known about temperature responses of individual key members of microbial communities decomposing SOC (Pietikäinen et al., 2005; Meier et al., 2010). For example, soil bacterial abundance may increase as a response to chronic soil warming coinciding with decreasing fungal abundance (Frey et al. 2008). On a much shorter time scale, Pietikäinen et al. (2005) observed that increasing temperature (above 30 °C) influenced fungal activity more negatively than bacterial activity, whereas an opposite temperature response of fungal and bacterial activities was observed at low temperatures (below 10 °C). Furthermore, soil microbial communities adapted to regional climatic conditions may respond distinctively to temperature variations. For example, cold adapted microbial communities may have a different optimum temperature, with cold adapted communities respiring more C at high temperatures (Bradford, 2013; Schindlbacher et al., 2015). Temperature can also indirectly affect microbial SOC decomposition. For instance, it controls microbial, substrate and/or enzyme diffusion and diffusion of enzymatic products by controlling soil moisture content (Steinweg et al., 2013; Ali et al., 2015). Microbial temperature response is

also affected by substrate availability through altered substrate sorption / desorption to organo-mineral complexes (Davidson and Janssens, 2006; Conant et al., 2011).

Investigation of microbial biomass and respiration response to temperature is also complicated by confounding effects of soil C quality (Devêvre and Horwáth, 2000; Hartley et al., 2008). For example, the pool of complex C compounds may be more strongly affected by temperature than the pool of rapidly degradable labile C (Knorr et al., 2005; Davidson and Janssens, 2006). Furthermore, C quality and temperature control the efficiency with which soil microbes utilize different C substrates. Frey et al. (2013) found a decrease in C utilization efficiency (C respired vs assimilated into biomass) with increasing temperature only for recalcitrant substrates. Steinweg *et al.* (2008) found the same temperature effect for cellobiose degradation. Carbon quality may also change microbial community composition to a greater extent than it changes the absolute amount of total microbial biomass (Cederlund et al., 2014). For example, the availability of complex C substrates has been shown to favor the dominance of fungal communities which degrade recalcitrant C compounds such as lignin (Yuste et al., 2011). Such an effect of substrate quality has also been observed under soil warming experiments, e.g., lack of labile substrate under soil warming supported fungal community, whereas increased labile substrate availability (via plants) shifted the dominance from fungal towards bacterial communities (Castro et al., 2010). Similarly, labile C rapidly increased soil respiration by stimulating an opportunistic bacterial community; specifically, members of *Gammaproteobacteria* and *Firmicutes* (Cleveland et al., 2007). However, this C-quality and microbial community relationship is not that straight forward and mixed substrate utilization responses of bacteria and fungi have been reported. For example, fast-growing fungi (yeasts) and specific bacterial taxa including *Actinobacteria* and *Proteobacteria*, both have been shown to utilize labile C compounds (Kramer et al., 2016). On the other hand, specific bacterial taxa such as *Bacteroidetes* were associated with decomposition of polymeric C substrate, for example, cellulose (Schellenberger et al., 2010). Based on their substrate preferences, predictable responses of diverse microbial taxa, for instance those exhibiting copiotrophic and oligotrophic behaviors, can make it possible to use them as indicators of soil trophic status (Cederlund et al., 2014; Fierer et al., 2007).

The aim of our study was, therefore, to better understand the temperature response of a diverse microbial community, specifically bacteria and fungi, utilizing substrates of different qualities,

which has rarely been tested simultaneously. The study was carried out in two geographically distinct regions and land-use types (bare fallow and soils influenced by vegetation). We assumed that each region and land-use type will be inhabited by distinct microbial communities adapted to region-specific climatic and edaphic conditions and to depleted labile C substrates in bare fallow soils. We, therefore, expected these specific microbial communities to show distinct responses to temperature and substrate quality variations. Additionally, we hypothesized that 1) microbial groups will show distinct temperature sensitivities in that fungal abundance will be high at low temperatures and bacteria will benefit from warm temperatures and that 2) addition of labile substrate will amplify the temperature response of bacterial abundance, whereas addition of complex substrates will pronounce fungal response to temperature changes. To accomplish the aim of this study, soil samples were amended with substrates varying in C quality (labile to moderately and strongly complex C compounds) and were exposed to different incubation temperatures. Changes in microbial community composition were determined by estimating phospholipid fatty acid (PLFA) and ergosterol content. In addition to the quantification of eight different bacterial groups using taxa-specific quantitative real-time PCR, bacterial and fungal abundances were evaluated using 16S rRNA gene and ITS fragment.

6.2 Materials and methods

Site description and soil sampling

Soil samples were taken from two agricultural sites in the Kraichgau and the Swabian Alb regions, both situated in Southwest Germany. The Kraichgau (48°55'7'' N, 8°42'2'' E; 100-400 m a.s.l) is an intensively used fertile loess region with a warm, dry climate, mean annual temperature (MAT) of 9.3 °C, and mean annual precipitation (MAP) between 720-830 mm. The dominant soil type in this region is Stagnic Cambisol on loess as parent material (WRB, 2007). In contrast, the Swabian Alb (48°31'7'' N, 9°46'2''E) is an extensively used grassland and cropland region with a more humid, cooler climate than the Kraichgau (MAT 7 °C, MAP between 800-1000 mm). The Swabian Alb is a karst plateau of Jurassic limestone with dominant soils classified as Calcic Luvisol, Anthrosol and Rendzic Leptosol (WRB, 2007). Some basic soil properties of both regions are found in Table S1.

In April 2013, soil samples (0-30 cm) were taken from two land-use types in each region; bare fallow and vegetated plots, five m^2 each. Bare fallow plots have been kept fallow since 2009 and managed by manual weeding and periodic spraying of glyphosate (Monsanto Agrar, GmbH, Germany). Vegetated plots were planted with winter wheat (*Triticum aestivum)* in the Kraichgau and winter rape (*Brassica napus*) in the Swabian Alb. Samples were sieved (< 2 mm) and stored at -24 °C until the start of the laboratory experiment. Results are reported based on oven dried soil (60 °C for 3 days).

Experimental design

We established the following treatments to test the effect of substrate quality on microbial activity at different temperatures: control (without any amendment), labile organic C as cellobiose (CB) (Sigma-Aldrich, St. Louis, USA), moderately complex C as xylan (from beech wood, SERVA Electrophoresis GmbH, Heidelberg, Germany), and strongly complex C as coniferyl alcohol (CA) (Sigma-Aldrich, St. Louis, USA). Substrate C was added at a rate of 5 % of the carbon content of soil samples under contrasting climatic and edaphic conditions (i.e. Kraichgau vs. Swabian Alb and vegetated vs. bare fallow plots). After thorough mixing, soil samples were filled into steel cylinders (100 cm^3, packed to their respective bulk densities) and placed in glass microcosms (volume: 480

ml). Microcosms were incubated at 5, 15, or 25 °C. Soil moisture content (at 60% WHC) was kept constant during the incubation period. Soil cores were incubated for either 7 days (CB) or 36 days (xylan and CA) depending on the decomposition rate of the added substrate. Two sets of controls were incubated, one for 7 days as control for the CB addition and the other for 36 days as control for the other amendments. Decomposition patterns of added C substrates were followed by measuring CO_2 production. Over the 36 days of the incubation experiment, soil respiration was measured seven times, on days 1, 3, 5, 7, 14, 28, and 36. Before taking gas samples, microcosms were closed with rubber stoppers. Fifteen ml headspace gas samples were collected by syringe at 0, 60 and 120 minutes after microcosm closure, then transferred to pre-evacuated exetainers (5.9 ml, Labco Ltd.). Concentrations of CO_2 in the collected samples were measured by gas chromatography (Agilent 7890A equipped with FID) and calibration was done by measuring known standard concentrations as described by Livingston and Hutchinson (1995). Cumulative flux of CO_2 was calculated as described by Poll *et al.* (2013). At the end of the incubation, soils were destructively sampled and stored at -24 °C until further analyses.

Analyses

Phospholipid Fatty Acids (PLFAs) and ergosterol

Four g (fresh weight) soil were used for extraction of PLFAs and their further transformation into fatty acid methyl esters (FAMEs) by alkaline methanolysis (Frostegård et al., 1991). Measurement of the extracted FAMEs was carried out by gas chromatography (AutoSystem XL, Perkin-Elmer Corporation, Norwalk, CT, USA) equipped with FID using helium as the carrier gas. For methodological details, see Kramer *et al.* (2013). Fatty acids including i15:0, a15:0, i16:0 and i17:0 were considered as gram-positive bacteria, and cy17:0 and cy19:0 were gram-negative. Total bacterial PLFAs included the gram-positive and gram-negative plus 16:1ω7, while 18:2ω6,9 represented the fungal PLFA. Total bacterial, fungal, and the following PLFAs comprised total PLFAs: 14:0, 15:0, 16:1ω6, 16:1ω5, 16:0, 17:0, 18:2ω6,9, 18:1ω9c, 18:3ω3, 18:1ω7, 18:1ω9t, 18:0, 20:4ω6, 20:5ω3, 20:3ω6, 20:2, 20:0, 22:0, and 24:0.

In addition to the 18:2ω6,9 PLFA, fungal biomass was also assessed by ergosterol content following a method described by Djajakirana *et al.* (1996) using one g fresh soil. For method specific details, see Kramer *et al.* (2012).

DNA extraction and quantitative PCR (qPCR) assay

DNA of 0.3 g soil was extracted using the FastDNA SPIN Kit for soil (MP Biomedicals, USA). Extracted DNA concentration was measured on a Nanodrop ND-2000 spectrophotometer (Thermo Scientific, USA). Quantification of 16S rRNA genes and fungal ITS fragment as well as eight taxa including *Alphaproteobacteria*, *Betaproteobacteria*, *Acidobacteria*, *Firmicutes*, *Gemmatimonadetes*, *Verrucomicrobia*, *Actinobacteria*, and *Bacteroidetes* was carried out with an ABI Prism 7500 Fast Real Time PCR System (Applied Biosystems, Germany). SYBR Green was used as the detection system. Reaction mixture included 4.473 µl H_2O, 0.75 µl of each primer, 0.027 µl T4GP32, 7.5 µl SYBR Green, and 1.5 µl DNA (5 ng μl^{-1}). PCR primers and thermal cycling conditions are given in Table S2.

Statistical analysis

All statistical analyses were performed using the R program for statistical computing – version 3.4.3 (R Core Team, 2017). Data homogeneity was tested with Levene's test for equality of variance. We used multi-factorial ANOVA to test the statistical significance of region, temperature, substrate amendment, and land-use (bare fallow vs vegetated) on microbial respiration, PLFAs and ergosterol content, 16S rRNA abundances, and fungal ITS fragments. Log transformation of 16S rRNA and fungal ITS data was performed to achieve normality. Least-squares means (lsmeans function of the lsmeans package; Tukey method for comparing a family of estimates) was used for pairwise comparisons (Russell, 2016). The relationship between the fungal PLFA and ergosterol was tested by Spearman's rank correlation (Bonferroni adjusted *P*-value). In order to determine how bacterial communities were affected by temperature and substrate amendments, principal component analyses were performed (R package stats) for each region using the relative abundances of bacterial groups. The relative abundance for each bacterial group was calculated as the ratio between copy numbers of the target group (estimated from individual group-specific qPCR assays) and the sum of copy numbers of the eight bacterial groups. Site scores of the first two principal components were further used in a multi-factorial ANOVA to test for temperature and substrate amendment effect. Significance was tested for $P < 0.05$ in all cases.

6.3 Results

Soil Respiration

Cumulative soil respiration was on average 38 % higher ($F_{1,96} = 137.41$, $P < 0.001$) in the Swabian Alb compared to the Kraichgau (Fig. 1). Land-use affected soil respiration ($F_{2,96} = 10.75$, $P < 0.001$) with bare fallow soils producing lower respiration than soils under vegetation influence (Figs. 1 and S1), however, the effect of land-use on soil respiration was smaller than the regional effect. Only minor differences in the temporal patterns of CO_2 production between bare fallow soils and soils under vegetation influence were observed. We therefore focus in the following only on CO_2 results from the vegetated soils. Temperature predominantly controlled microbial respiration response in soils under vegetation influence by generally increasing cumulative respiration in all substrate treatments and in both regions with maximum respiration measured at 25 °C ($F_{2,48} = 91.51$, $P < 0.001$; Fig. 1). In the Swabian Alb, substrate addition led to increased respiration at all temperatures and with all substrates when compared to un-amended controls ($F_{3,24} = 87.56$, $P < 0.001$). In contrast, substrate addition generally increased cumulative respiration in the Kraichgau only at 15 and 25 °C with xylan addition showing the largest effect when compared to un-amended controls (substrate × temperature, $F_{6,24} = 3.10$, $P < 0.05$; Fig. 1). During the first week of incubation, CB induced higher respiration than xylan at all incubation temperatures in both regions, and almost no difference was observed between CA and un-amended controls (Fig. S2).

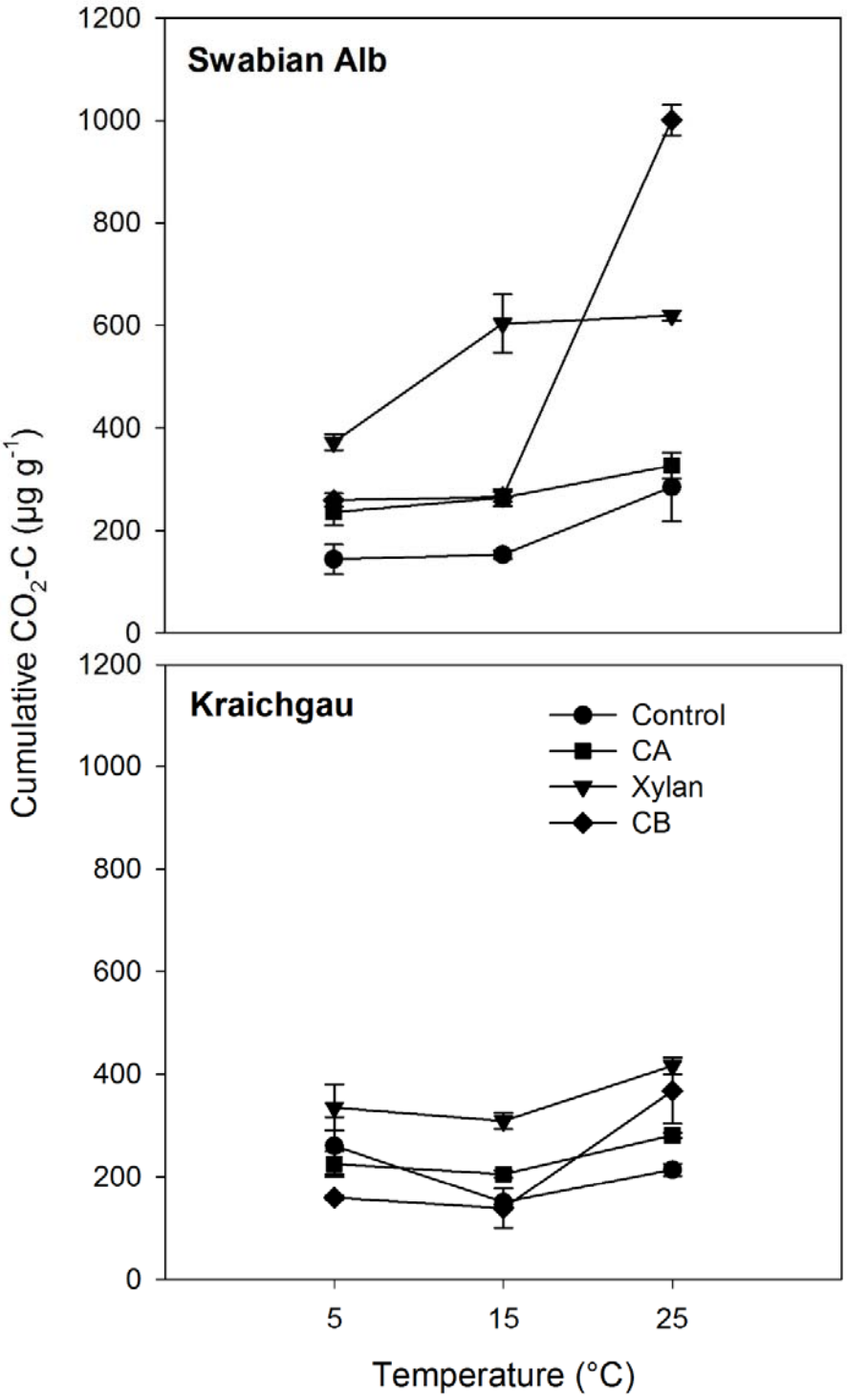

Fig. 6.1 Microbial respiration flux (cumulative CO_2-C) measured from vegetated soils of the Swabian Alb and the Kraichgau regions under different C substrate amendments. CA = coniferyl alcohol, CB = cellobiose. Bars indicate standard error (n = 3).

PLFA content

Control samples, incubated for either 7 days or 36 days, showed similar trends and were not statistically different from one another. We therefore show PLFA data only for the controls after 36 days' incubation at the respective temperatures.

Both regions differed from each other with respect to the size of microbial biomass content; the Swabian Alb had 51 % higher total PLFA content than the Kraichgau ($F_{1,107} = 1327.65$, $P < 0.001$; Fig. 2 a, b). The difference in extracted PLFAs between bare fallow soils and soils influenced by vegetation was smaller than the regional effect, with vegetated soils having 43 % higher total PLFA content than bare fallow soils ($F_{2,107} = 550.62$, $P < 0.001$, Figs. 2 a, b and S3 a, b). Substrate addition generally led to increased total PLFA content across all temperatures, regions, and land-uses with CB addition exhibiting the largest effect ($F_{4,107} = 426.10$, $P < 0.001$). The temperature effect was smaller than the substrate addition effect and negatively influenced total PLFAs with lowest values recorded at highest temperatures ($F_{2,107} = 237.52$, $P < 0.001$; Figs. 2 a, b and S3 a, b). The lipid profiles showed decreasing unsaturation that depended upon substrate amendment in both regions with increasing temperature (temperature × substrate × region; $F_{8,107} = 2.18$, $P < 0.05$, Fig. S9).

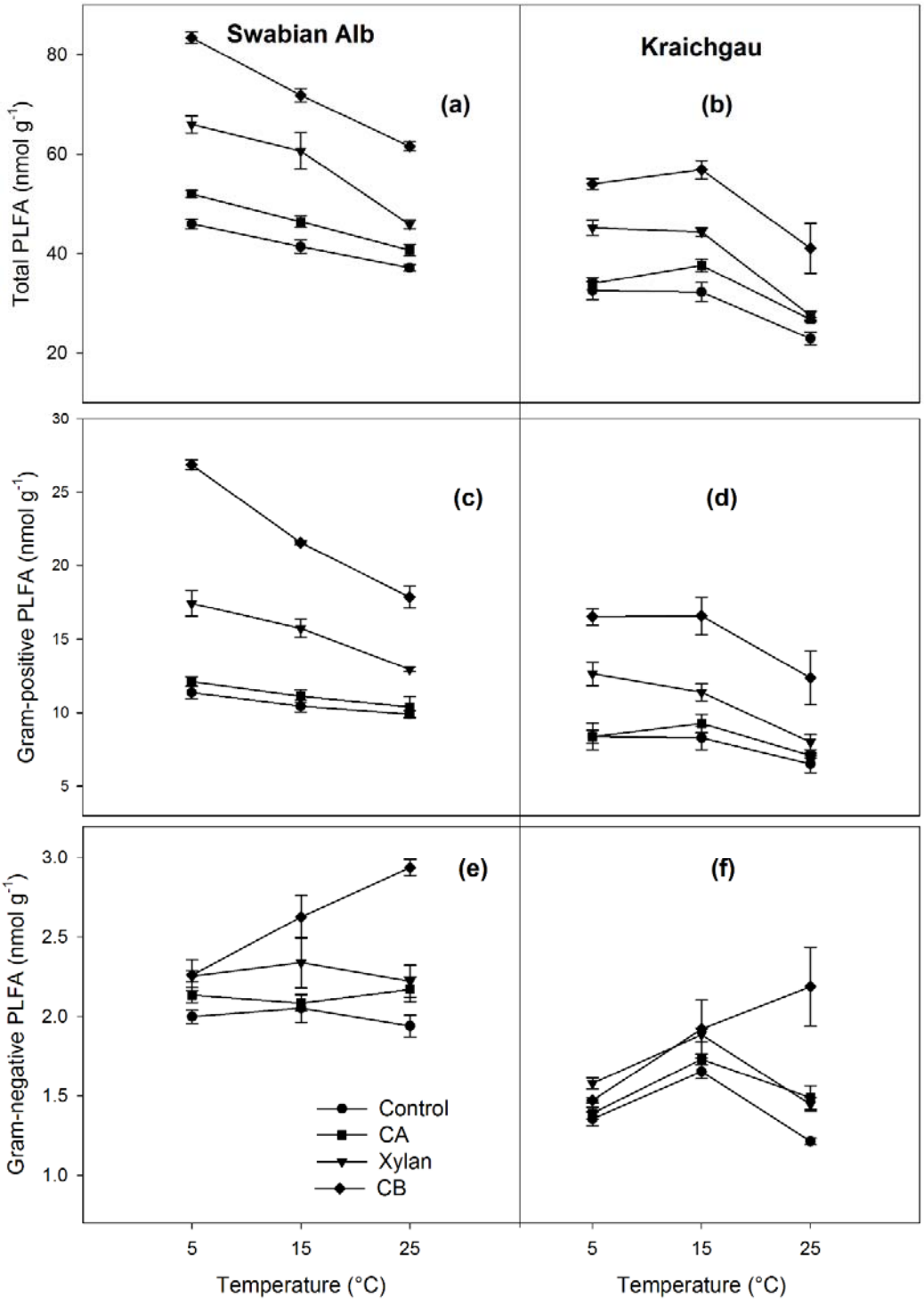

Fig. 6.2 Shifts in total, gram-positive, and gram-negative bacterial PLFAs of vegetated soils in response to incubation temperature and C substrate of varying qualities. Left panels represent PLFAs of the Swabian Alb (a, c, e) and right panels represent PLFAs of the Kraichgau (b, d, f). Bars indicate standard error (n = 3).

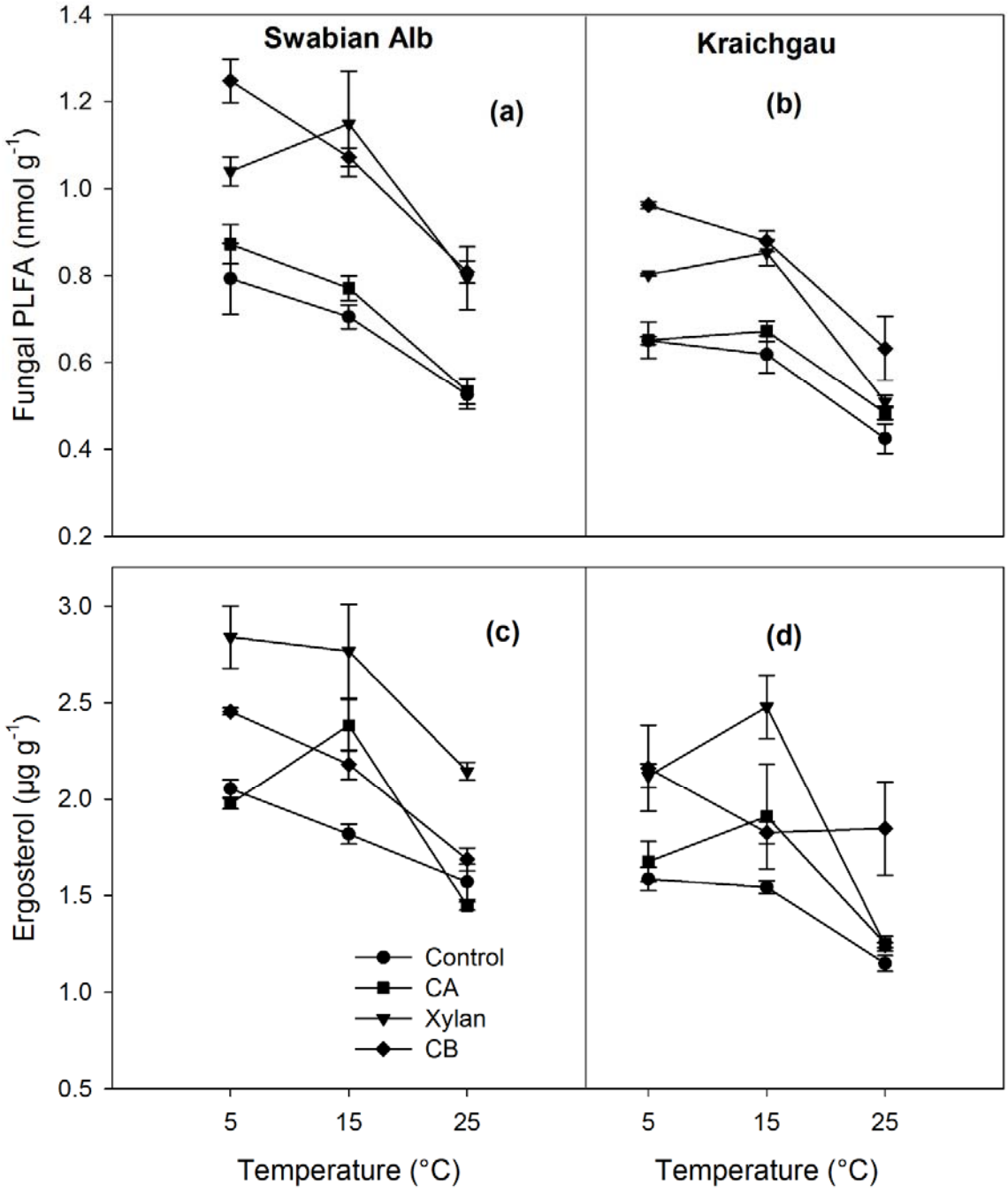

Fig. 6.3 Fungal PLFAs and ergosterol contents measured in vegetated soils of the Swabian Alb (a, c) and the Kraichgau (b, d) un-amended and amended with C substrates of varying qualities after incubation at three different temperatures. Bars indicate standard error (n = 3).

Abundance of soil bacterial groups were more affected by the regional than the land-use effect with higher PLFA contents in the Swabian Alb than in the Kraichgau ($F_{1,107} = 716.41$, $P < 0.001$; $F_{1,107} = 829.40$, $P < 0.001$, respectively; Figs. 2 and S3 c, d, e, f). Vegetated soils showed higher contents of gram-positive and gram-negative bacterial PLFAs than the bare fallow soils ($F_{2,107} = 163.30$, $P < 0.001$; $F_{2,107} = 24.48$, $P < 0.001$, respectively; Figs. 2 and S3 c, d, e, f). Substrate addition affected abundance of gram-positive and gram-negative bacterial PLFAs more strongly than the rising temperature. PLFAs of gram-positive bacteria decreased with increasing temperature in both regions (Fig. 2 c, d). There was only one exception to this general trend: the use of CA by gram-positive bacteria was not influenced by temperature (Fig. 2 c, d). Consequently, the interaction between temperature × substrate ($F_{8,107} = 17.57$, $P < 0.001$) was significant. Total bacterial PFLAs showed similar trends with respect to temperature and substrate amendments as those of gram-positive bacteria (data not shown). Like gram-positive bacteria, gram-negative bacterial PLFAs generally increased after substrate addition at all temperatures with the strongest increase recorded after CB addition ($F_{4,107} = 46.41$, $P < 0.001$; Fig. 2 e, f). PLFA content of gram-negative bacteria increased from 5 to 15 °C, irrespective of substrate quality. For soils amended with CB, gram-negative PLFAs further increased with increasing temperatures in both regions and land-uses (substrate × temperature; $F_{8,107} = 12.15$, $P < 0.001$; Figs. 2 and S3 e, f).

Like bacterial biomass, the fungal PLFA content was also significantly higher in the Swabian Alb than in the Kraichgau ($F_{1,107} = 178.19$, $P < 0.001$) and was significantly increased in soils influenced by vegetation compared to bare soils ($F_{2,107} = 363.09$, $P < 0.001$, Figs. 3 and S4 a, b). Land-use had the strongest effect on fungal PLFAs and temperature and substrate quality affected fungal PLFAs almost equally (Fig. 3 and S4). Fungal PLFAs were highest in CB and xylan amended soils ($F_{4,107} = 147.50$, $P < 0.001$), whereas after CA addition fungal PLFAs increased only slightly. In general, increasing temperature reduced fungal biomass; lowest fungal PLFAs were recorded at 25 °C and fungal PLFAs increased from 5 to 15 °C in most cases after the addition of xylan and CA (temperature × substrate; $F_{8,107} = 9.89$, $P < 0.001$; Fig. 3 a, b).

Ergosterol

A significant correlation ($r = 0.89$, $P < 0.05$) was found between the two fungal biomarkers investigated in our study; ergosterol and fungal PLFAs. Land-use affected ergosterol content most

strongly, and like fungal PLFAs, temperature and substrate quality equally affected ergosterol contents (Figs. 3 and S4 c, d). Soils of the Swabian Alb had 20 % more ergosterol content than soils of the Kraichgau ($F_{1,117}$ = 130.07, P < 0.001). Ergosterol content also differed between investigated land-uses with bare fallow soil having significantly lower (-72 %; $F_{2,117}$ = 725.16, P < 0.001) ergosterol content than soils influenced by vegetation. In most cases, substrate addition increased ergosterol content across all temperatures, regions, and land-uses with xylan addition showing the largest effect ($F_{4,117}$ = 126.00, P < 0.001, Figs. 3 and S4 c, d). After CA addition, a clear increase in ergosterol content was recorded at 15 °C (substrate × temperature; $F_{8,117}$ = 12.75, P < 0.001; Fig. 3 c, d). This effect of complex substrate addition on ergosterol content was greater than that observed for the fungal PLFAs, where no such increase was recorded. Lowest ergosterol values were recorded at 25 °C in both regions and land-uses, as also observed for the fungal PLFAs ($F_{2,117}$ = 193.16, P < 0.001; Fig. 3). Temperature effect on added substrate quality was distinct at 5 and 15 °C but not at 25 °C, where ergosterol content converged back to the control values with an exception of CB in the Kraichgau and xylan in the Swabian Alb (region × temperature × substrate; $F_{8,117}$ = 3.04, P < 0.01; Fig. 3 c, d).

Gene abundances of different bacterial taxa

Bare fallow and soils influenced by vegetation showed, for the most part, similar trends in response to temperature and substrate amendments (Figs. 4, S5). We therefore focus here only on the results of soils influenced by vegetation. Temperature and substrate amendment both affected 16S rRNA gene abundance with substrate addition effect being larger than the temperature effect (Figs. 4 and S5). Increasing temperature generally increased the abundance of 16S rRNA after CB and xylan additions, and this was most pronounced in the Swabian Alb (Fig. 4). In contrast, soils amended with CA showed a much smaller temperature response, and their highest 16S rRNA abundances after incubation were observed at 15°C (region × temperature × substrate, $F_{8,54}$ = 2.42, P < 0.05; Fig. 4). The fungal ITS fragment, in contrast to 16S rRNA gene abundance, was generally lowest in abundance at 25 °C (region × temperature $F_{2,54}$ = 4.10, P < 0.05), with substrate addition effect being larger than the temperature effect (Fig. 4). In the Kraichgau, the temperature response of the fungal ITS fragment to individual substrate amendments differed between xylan and CA, with a peak at 15 °C, while in the CB amendment they were highest in abundance at 5°C (temperature × substrate $F_{8,27}$ = 2.67, P < 0.05; Fig. 4). In the Swabian Alb, a similar pattern was observed,

although less pronounced for xylan and CA and more pronounced for CB (temperature × substrate $F_{8,27} = 1.99$, $P = 0.09$; Fig. 4).

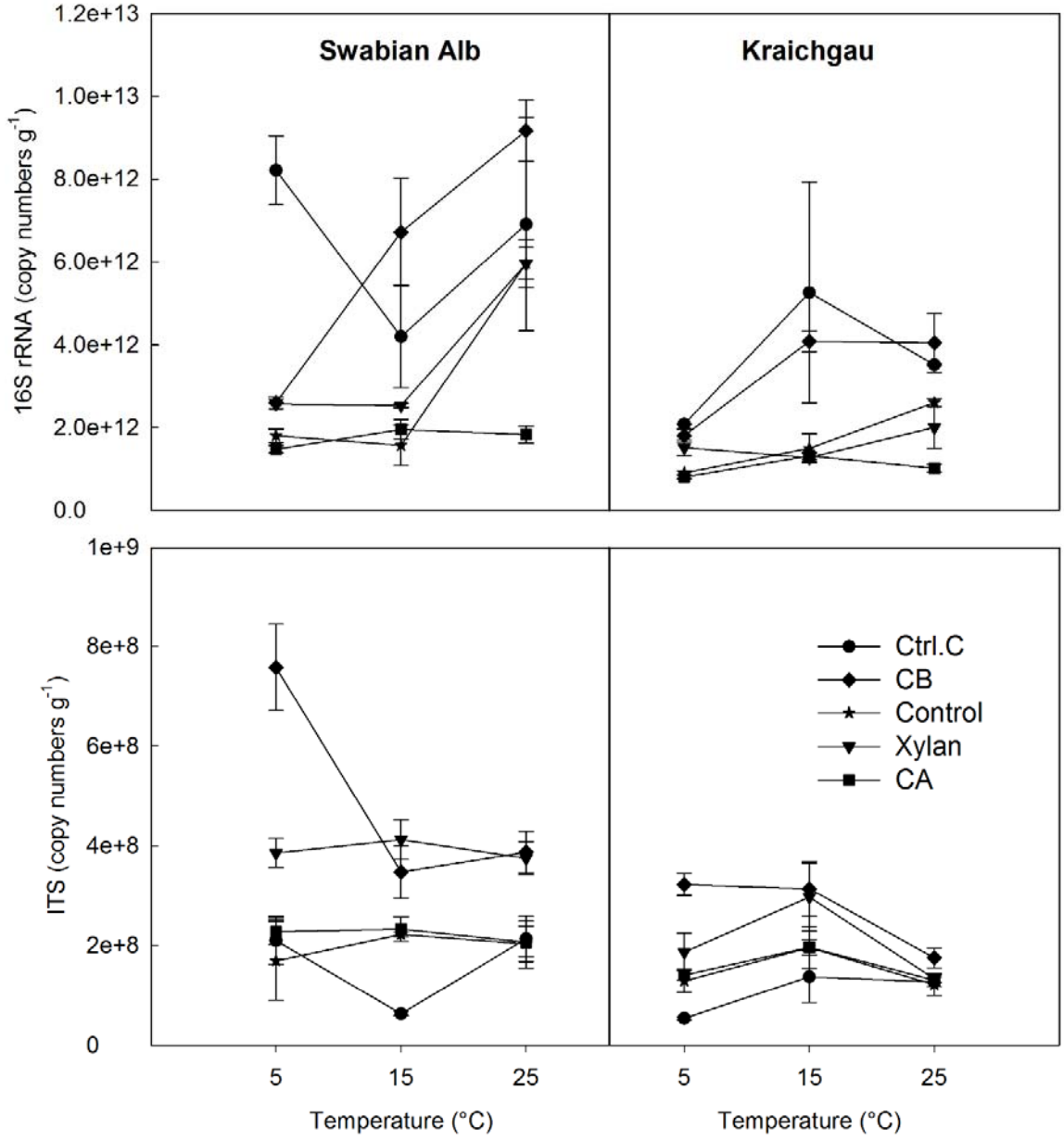

Fig. 6.4 16S rRNA gene abundance and quantities of fungal ITS fragment from the Swabian Alb and the Kraichgau vegetated soils as affected by temperature and different C substrate qualities. Bars indicate standard error (n = 3). Ctrl.C are un-amended controls after 7-days' incubation and "Control" are un-amended controls after 36-day incubation.

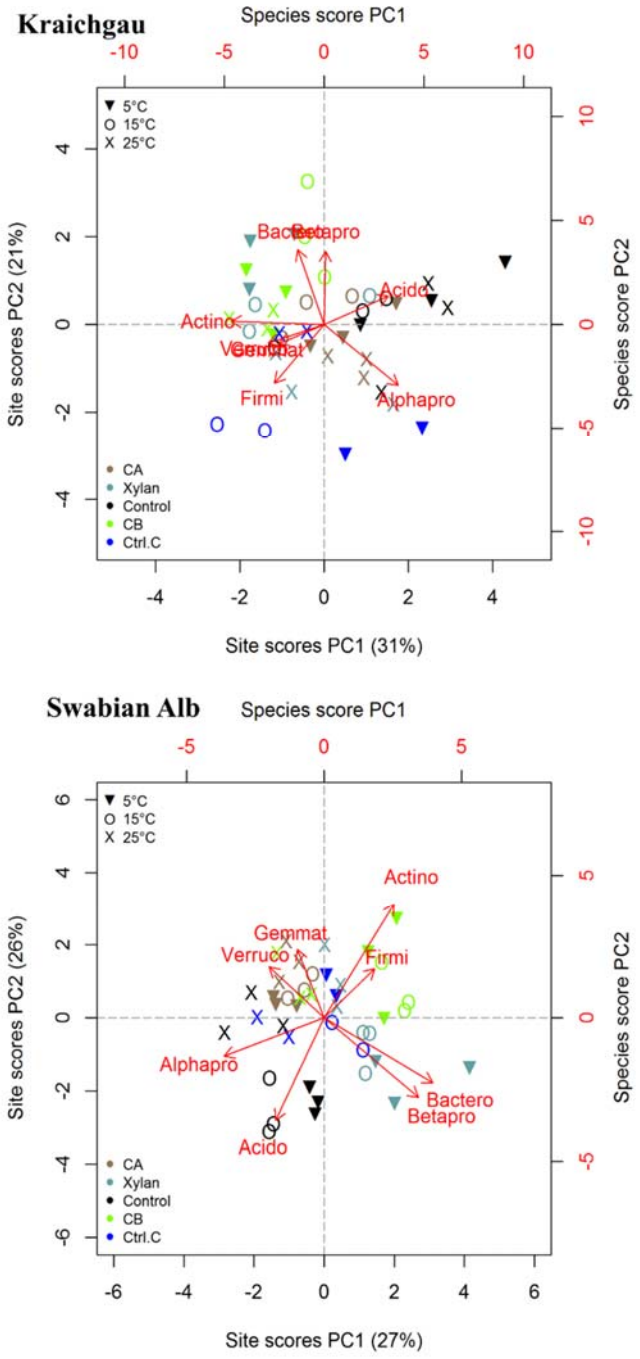

Fig. 6.5 Results of PCA analysis for bacterial community composition from vegetated soils as affected by different temperatures and substrate amendments. Species scores are in the form of arrows with different bacterial taxa (Actino = *Actinobacteria*, Alphapro = *Alphaproteobacteria*, Betapro = *Betaproteobacteria*, Acido = *Acidobacteria*, Bactero = *Bacteroidetes*, Gemmat = *Gemmatimonadetes*, Firmi = *Firmicutes*, Verruco = *Verrucomicrobia*). For legend, see Figure 6.4.

Of eight microbial taxa, *Alphaproteobacteria*, *Acidobacteria,* and *Actinobacteria* were the three most abundant in all substrate amendments and at all incubation temperatures with mean relative abundances of up to 19, 26, and 37 % in the Swabian Alb, and 32, 28 and 31 % in the Kraichgau, respectively (Figs. S7 and S8; for absolute abundances of respective taxa, see Table S3). Principal component analyses (PCA) were carried out to discern the influence of temperature and substrate quality on soil bacterial community composition. Together, the first two principal components (PCs) explained 52 % and 53 % of the observed variance in bacterial taxa for soils influenced by vegetation in the Kraichgau and the Swabian Alb, respectively, which was lower than the explained variance for bare fallow soils in both regions (Figs. 5, S6). In both regions, substrate amendment effect was larger than temperature effect. Specifically, in the Kraichgau vegetated soils, no temperature effect was found along PC1 but there was a slightly significant interaction between temperature and substrate amendment (temperature × substrate $F_{8,27} = 2.56$, $P < 0.05$). *Actinobacteria* and *Acidobacteria* had opposite loadings along the first PC; *Actinobacteria* associated more strongly with CB and *Acidobacteria* associated more strongly with un-amended controls and CA (Fig. 5). Significant temperature and substrate effects were also visible along PC2 (21 % explained variance) in the Kraichgau (temperature × substrate $F_{8,27} = 6.84$, $P < 0.001$) where, in CB amended soils, samples at 15 °C diverged from those at 5 and 25 °C (Fig. 5). Similarly, temperature and substrate significantly affected bacterial community distribution along the first two principal components in the Swabian Alb (temperature × substrate, for PC1 $F_{8,27} = 5.16$, $P < 0.001$; for PC2 $F_{8,27} = 4.49$, $P = 0.001$) with temperature separating CB along PC1, and CA, xylan and their respective controls along PC2 (25 °C separated from 5 and 15 °C; Fig. 5). *Actinobacteria* and *Acidobacteria* had opposite loadings along the second PC.

6.4 Discussion

Despite the critical role soil microorganisms play in controlling terrestrial C cycle processes and its feedbacks to climate change, how soil microbes change in their abundance and respiration responses to temperature and SOC quality remains unclear (Hartley et al., 2008). On one side, temperature change has been associated with shifts in microbial community composition (Bradford, 2013) and on the other side, temperature sensitivity of microbial SOC decomposition has been associated with C quality and its availability to microbial decomposers (Davidson and Janssens, 2006; Moinet et al., 2018). Here, we present results from a microcosm study where the specific temperature responses of microbial groups in the presence of three substrates of varying quality (CB, xylan, CA) were investigated in two regions and land-use types. Our study mimics complex environments where microbial abundance is not only affected by environmental conditions, but also depends upon competition between microbial groups for available resources, such as soil bacteria and fungi. When a certain microbial group is more abundant in response to the addition of a certain substrate, compared to un-amended controls, we assumed this as an indication of utilization of this specific substrate. Nevertheless, other mechanisms such as priming effect may also contribute, to some extent, to the changes in abundance of specific microbial groups (Zhang et al., 2013).

We expected that the relative differences in climatic and edaphic properties of both regions and the differences in the organic matter quality of bare fallow soils and soils influenced by vegetation would select for specific soil microbial communities, which would in turn respond in distinctively different ways to our experimental treatments. Contrary to our expectations, we observed differences in microbial abundances only in absolute terms: C rich soils of the Swabian Alb had higher microbial abundances than the Kraichgau (e.g., see Figs. 2 and S3), and soil influenced by vegetation had higher microbial biomass than bare fallow soils, but the response to temperature change and substrate amendments did not differ. Therefore, region and land-use seem to have much smaller impacts on microbial response to temperature than expected.

Temperature sensitive response of soil microbial biomass

Rising temperatures are largely predicted to stimulate the decomposition of SOC, and soil microorganisms and their community composition, e.g., bacteria and fungi, are important in this

context due to their role as the mediators of carbon-climate feedback (Allison et al., 2010; Jagadamma et al., 2014). Therefore, understanding the temperature sensitive responses of microbial abundances and respiration is important to understand changes in C fluxes in the context of climate warming (Classen et al., 2015; Alster et al., 2016). In our study, temperature was the main factor affecting soil microbial respiration response. Temperature negatively influenced total microbial biomass (total PLFAs) irrespective of land-use and region, with the lowest PLFAs usually measured at highest incubation temperatures (Figs. 2 a, b and S3 a, b). Such a decrease in microbial biomass with warming has been reported by others (Zogg et al., 1997; Waldrop and Firestone, 2004; Frey et al., 2008; Wu et al., 2010) and attributed to increased metabolic stress at high temperatures (Zogg et al., 1997). We witnessed a strong increase in a metabolic stress indicator with increasing temperature, as indicated by the ratio of the fatty acid cy17:0 to its metabolic precursor 16:1ω7 (Schindlbacher et al., 2011), which was consistent in all C substrate amendments including un-amended controls (data not shown). Decreased total PLFAs with increasing temperatures coincided with an opposite microbial cumulative respiration response, especially in CB amended soils (Figs. 1 and S1), hinting at a metabolic shift to catabolic processes. Temperature increase forces microbes to spend available resources more on maintenance than on biosynthesis, a trend which has previously been observed (Steinweg et al., 2008; Allison et al., 2010; Frey et al., 2013; Tucker et al., 2013). Observed decrease in microbial biomass with increasing temperature may also be associated with specific microbial communities present in both study regions at the time of soil sampling. Average soil temperatures in the Kraichgau and the Swabian Alb, one month before soil sampling, were 3.3 °C and 1.3 °C, respectively, which may indicate support for the presence of psychrophilic microorganisms. Psychrophiles tend to have an increased proportion of unsaturated fatty acids (Nedwell, 1999) and changes in microbial PLFA composition with increasing temperature, for instance, decreases unsaturated fatty acids (Marr and Ingraham, 1962). As this was observed in our study (Fig. S9), it possibly corresponds to the energy-requiring biosynthesis of saturated fatty acids (Zogg et al., 1997). Thus, it may be that with increasing temperature, certain members of microbial communities may not be able to keep up with the energy demand, leading to metabolic stress or death (Petersen and Klug, 1994). In our study, soil fungal biomass was negatively affected by increasing temperature, an effect consistent through all fungal biomarkers (Figs. 3 and 4). Furthermore, increasing temperature from 5 to 15 or 5 to 25 °C affected fungal biomass more adversely than bacterial biomass (gram-positive bacteria; e.g., Figs. 2 and 3

a, b). This difference in the strength of temperature-sensitive response may indicate that fungi are more sensitive to temperature variations than bacteria due to their thermal adaption strategies or physiological differences (Alster et al., 2018). Such temperature-related biomass response of soil microbial groups partially confirms our initial hypothesis that low temperature may support fungal biomass more than bacterial biomass. However, we could not confirm our hypothesis, that bacteria will benefit from high temperatures, as the abundance of gram-positive bacteria decreased with increasing temperatures and gram-negative bacteria showed a temperature optimum at 15°C. In the context of climate warming, such a response of microbial groups may lead to shifts in fungi/bacteria ratios towards higher bacterial biomass, as has been observed in earlier studies (Pietikäinen et al., 2005). Lipson et al. (2002) also found a more positive effect of low winter temperatures on fungi than bacteria leading to a higher fungi/bacteria ratio. However, a lower substrate utilization efficiency and higher biomass turnover rate for bacteria than for fungi (Allison et al., 2005; Gunina et al., 2017) may significantly affect C cycling and storage in soil systems following climate warming (Six et al., 2006) and may also provide positive feedback to climate change, at least at the short-term scale investigated in our study.

SOC quality controls the temperature sensitive response of microbial key players involved in SOC decomposition

Substrate quality is expected to be among the main drivers of microbial community composition (Goldfarb et al., 2011). At the same time, it may also modify the temperature response of microbial groups decomposing SOC. Bacterial abundance, as assessed by 16S rRNA gene abundance, responded positively to added substrates at high temperatures in the order CB > xylan > CA (Fig. 4), corresponding to our hypotheses that bacterial biomass may increase with warming and labile C substrate availability. Gunina et al. (2017) also found that bacteria were more active in labile C utilization (glucose) than fungi. The relative difference in bacterial biomass between added substrate qualities may be explained by 16S rRNA copy numbers per genome (see discussion below). The number of rRNA genes correlates with the rate at which phylogenetically diverse bacteria respond to substrate availability (Klappenbach et al., 2000). Therefore, having more than one 16S rRNA copy per cell could provide a competitive advantage that translates to an increased biomass for a particular community when suitable substrates become available.

The temperature response of gram-positive bacterial abundance basically did not change with substrate addition, but the strength of the response was confounded by substrate quality. The addition of coniferyl alcohol induced only small changes, whereas the addition of cellobiose and xylan induced a pronounced increase in the abundance of gram-positive bacteria at low temperatures (Fig. 2 c, d). Similarly, a temperature induced decline in gram-positive bacterial abundance in soils amended with complex substrate (maize residues with C/N ratio ~ 52 incubated at 10 or 30 °C) has been reported by Bai et al. (2017a). The pattern observed in our study may suggest a food preference of gram-positive bacteria for organic C of different qualities. Consistent with our results, Wang et al. (2015) found higher gram-positive bacterial abundances in forest soils amended with substrate of high quality (leaves, low C/N ratio) than soils amended with low quality substrate (maize stalk, high C/N ratio). Other studies have reported mixed responses of gram-positive bacterial biomass to changing temperature and substrate quality (Waldrop and Firestone, 2004; Hopkins et al., 2014; Wei et al., 2014). Since gram-positive bacteria are known to react to a variety of substrates ranging from easily available to more complex compounds (Müller et al., 2016), substrate quality may change the feeding behavior of gram-positive bacteria, hence their abundance in the context of climate warming.

Gram-negative bacteria, on the other hand, responded differently to temperature and substrate amendments than gram-positive bacteria (Figs. 2 and S3). In most cases, there was an increase from 5 to 15 °C and a decrease from 15 to 25 °C. The addition of CB increased their abundance up to 25 °C. Gram-negative bacteria generally exhibit copiotrophic behavior; they grow in high-quality resource-rich conditions (Fierer et al., 2007; Fanin et al., 2014; Zhang et al., 2016). Therefore, our observed temperature response of gram-negative bacteria to the CB amendment, which represents relatively high-quality C substrate, fits this pattern. Furthermore, high turnover rates of copiotrophic gram-negative bacteria (Fierer et al., 2007) at high temperatures could also explain the observed increase in cumulative respiration in CB amended soils of both study regions (Figs. 1 and S1). However, these results must not be over interpreted, as the shorter incubation duration for CB amended soils may explain why gram-negative bacterial PLFAs responded differently than in xylan and CA amended soils at 25 °C. At high temperatures, especially at 25 °C, incubation time may have been an important factor due to faster exhaustion of available substrate, since C mineralization rates are known to be much higher at high than low temperatures (Feng and Simpson, 2009; Tang et al., 2018).

Furthermore, our results showed that fungi did not have any specific food preference for either labile or complex substrates, opposite to our initial hypothesis of fungi utilizing complex substrate. Fungal biomass increased in soils amended with labile substrate (CB) either equally or even stronger than in soils amended with more complex substrates (xylan and CA) (Fig. 3). Utilization of relatively simple compounds by fast-growing opportunistic fungi may explain these observations (Meidute et al., 2008). A clear separation of fungi utilizing complex substrates and bacteria primarily utilizing simple substrates was also not verified by Kramer *et al.* (2016). This opportunistic behavior of fungi may have important implications for C cycling due to their role in soil C stabilization dynamics. Nevertheless, fungal biomass and respiration response to temperature variation was confounded by substrate quality; CB addition did not alter temperature response of fungi, whereas xylan and CA amendment shifted the fungal temperature optima from 5 °C to 15 °C (Fig. 3). These results may suggest possible coupling of fungal life strategies with substrate use of different qualities. It may be that fungi which use complex substrates, for instance lignin, have higher temperature optima than fungi using simple sugars. Kerry (1990) compared the temperature behavior of leaf and litter colonizing fungi and concluded that phyllospheric fungi had a lower temperature optimum than saprotrophic fungi feeding on more complex material.

Response of bacterial taxa to temperature and substrate quality variations

To decipher the soil microbial community's abundance and respiration responses to changing temperature and C resource quality, we investigated eight bacterial taxa using a quantitative PCR approach (Figs. 5 and S6). Investigated taxa in our study are among the most dominant found in soil systems at the global scale, as recently suggested by Delgado-Baquerizo *et al.* (2018). Therefore, by targeting these taxa, we sought to identify a great diversity of bacteria and include their response to our experimental amendments. PCA projections using taxon relative abundance confirmed significant interactive controls of temperature and substrate quality over microbial community composition in both study regions and analysis of variance further indicated the substrate effect alone being the largest (Figs. 5 and S6). Association with temperature has been shown previously, even at the global scale, to be among the best predictors of microbial abundance (Delgado-Baquerizo et al., 2018). However, associating changes in microbial community composition due to, for example, temperature or resource quality through use of taxa specific information can be challenging. Microbial processes, such as decomposition of organic C of

varying complexity, may be the result of myriad integrated metabolic pathways that can be carried out by a broad range of taxa (Fierer, 2017). That could be one reason that no clear trends were seen for a temperature- and / or substrate-specific taxon response in our study. Therefore, distinguishing different taxa based on some shared life strategies for survival under specific environmental conditions, for example copiotrophic vs oligotrophic, may be more meaningful. We would expect a relatively higher association of oligotrophs with the soils amended with complex substrates (CA and xylan), but expect copiotrophs to be more strongly associated with the soils amended with relatively labile substrates (CB). Consistent with these interpretations, taxa which represent oligotrophs such as *Alphaproteobacteria* and *Acidobacteria* (Goldfarb et al., 2011) were associated more with the un-amended controls and complex C substrates (CA or xylan), whereas taxa exhibiting copiotrophic behavior such as *Actinobacteria* (Goldfarb et al., 2011) were associated more with CB amended soils (Figs. 5 and S6). Copiotrophic bacteria, including *Betaproteobacteria* and *Bacteroidetes* (Goldfarb et al., 2011), were also associated with CB amended soils along PC2 in the Kraichgau; however, in the Swabian Alb, they were associated with xylan (a somewhat complex substrate) and un-amended controls incubated for 7 days. This overlapping utilization of labile with rather complex substrates, despite regional climatic and geological differences between study regions, could be attributed to non-specific or broad substrate utilization spectra of these taxa. Fierer *et al.* (2007) argued against distinct categorization of *Betaproteobacteria* and *Bacteroidetes* as copiotrophic or oligotrophic due to evidence that certain members of these taxa could behave otherwise. Therefore, further research is needed to improve our understanding of the metabolic plasticity of soil bacteria in their ability to decompose substrates of different qualities to better predict climate-induced temperature variations.

Different microbial biomarkers vary in their response to temperature and substrate quality

The bacterial community's response to changing temperature, as measured by 16S rRNA gene abundance, was opposite that observed in the bacterial PLFA biomarkers (Figs. 2 and 4). Even though the variation between replicate samples could be higher in a qPCR compared to PLFA assay, these two methods of microbial biomass estimation have been positively correlated to each other (Zhang et al., 2017; Orwin et al., 2018). The opposite behavior of bacterial PLFAs and gene abundances may have been due to differences in gene copy numbers and different percentages of relic DNA of specific bacterial taxa. The copy numbers of 16S rRNA from different bacterial

genomes vary from one to as many as 17 per genome (Klappenbach et al., 2000; Větrovský and Baldrian, 2013; Stoddard et al., 2015). We observed an increase in the absolute abundances of different taxa at 25 °C (compared to un-amended controls, Table S3) which are known to have more than one 16S rRNA gene copy; for instance, *Proteobacteria*, *Fimicutes*, and *Actinobacteria* (Stoddard et al., 2015), which was not reflected in the bacterial PLFA data. An alternative explanation could be differences in the ratio of DNA extracted either from active cells or from extracellular DNA (relic or historical DNA) of different taxa (Fierer, 2017). Relic DNA, which is found in cells with compromised cytoplasmic membranes, may increase bacterial diversity estimates by more than 40% (Carini et al., 2016; Fierer, 2017). Nevertheless, discrepancy between microbial PLFA and 16S rRNA gene analysis might also be caused by stress-induced changes in microbial PLFA pattern with soil warming (see discussion above), which may not be reflected in 16S rRNA analysis. For example, Schindlbacher *et al.* (2011) observed no significant warming effect on microbial community composition but warming soil for 5 years led to increased abundance of PLFA stress biomarkers. Therefore, soil warming studies performed at a short time scale may produce different results due to choice of biomarker of microbial community composition. In comparison, studies performed at a longer time scale may be independent of biomarker bias, for example, due to adaptation of microbial community to temperature and altered substrate availability and quality (Bradford, 2013).

The two fungal markers (ergosterol and fungal PLFA) were not comparable after the addition of CA: whereas ergosterol content increased under this condition at 15 °C, the fungal PLFA did not increase (Figs. 3 and S4). Differences in coverage of specific fungal groups by the two biomarkers could explain these results: ergosterol is mainly produced by higher fungi, for instance, *Ascomycetes* and *Basidiomycetes* and these groups are also typically known to decompose complex substrates such as lignin (Weete and Gandhi, 1997; Hanson et al., 2008). Other classes of fungi, such as. *Zygomycetes*, include fast-growing organisms. Some of these may lack ergosterol (e.g. *Mortierellales*), vary in their fungal fatty acid profiles (linoleic acid) and decompose relatively labile C (Stahl and Klug, 1996; Weete and Gandhi, 1999; Hanson et al., 2008; Richardson, 2009; Ruess and Chamberlain, 2010). Similar correlations frequently found in the literature between the two biomarkers (e.g. by Müller et al. 2016) may hold true only for ecosystems with similar substrate quality and temperature variation.

6.5 Conclusions

It is crucial to understand how soil microorganisms respond to environmental changes because of their importance in carbon cycling. Our aim was to test whether soil temperature, substrate quality, and their interactive effects modify the abundance of soil bacteria and fungi, the key players involved in SOC decomposition, in geographically different regions and under different land-uses. The much larger impact of temperature and substrate quality rather than region and land-use on microbial abundance identified the first two factors as having important implications for understanding the response of C cycling in soils. Strong stress response of soil bacteria and fungi to increasing temperature that resulted in high respiration, but reduced biomass indicates that climate warming could provide positive feedback to climate change at a short time scale. Temperature increase also affected fungal biomass more negatively than the bacterial biomass further complementing the positive feedback response considering the fungal role in SOC stabilization and C sequestration. However, the strength of this feedback would depend upon the strength of respiration increase *vs* biomass reduction. At a longer time scale, reduced microbial biomass may results in reduced SOC decomposition and, together with possible adjustments in microbial carbon use efficiencies (Frey et al., 2013; Tucker et al., 2013) reduce the intensity of this positive feedback. Our results provide evidence that substrate quality partly controls the temperature response of soil microbial communities. In total, the observed complex interactions between different groups of soil microorganisms, substrate quality, and temperature, gave evidence that the response of carbon fluxes to climate change will be strongly driven by shifts in microbial community's functionality.

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7 Controls on microbially regulated soil organic carbon decomposition at the regional scale

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Key words: Soil respiration; Q_{10}; Enzyme activity; Landscape scale; Regional scale

Abstract

Even small changes in microbial decomposition rates of soil organic carbon (SOC) at the regional scale have the potential to modify land-atmospheric feedbacks at the global scale. Limited understanding of the regulation of microbial driven processes has led to major uncertainty in global SOC estimates. Therefore, to better understand the large scale processes controlling SOC dynamics, we examined the influence of SOC quantity, quality, and soil physical and biochemical properties on soil basal respiration and of the temperature sensitivities (Q_{10}) of soil respiration and enzymes (β-glucosidase and xylanase) at two scales: landscape (two individual areas, each approximately 27 km^2) and regional (pooled data of both areas). Soil samples (0-30 cm soil depth) originated from 41 agricultural sites distributed over two areas in southwest Germany differing in climatic and geological conditions. We used a two-step data analysis procedure; variable selection through random Forest regression, followed by shortlisting of significant explanatory variables using linear mixed-effect models. Microbial biomass regulated soil basal respiration at both scales, whereas soil C:N ratio played an important role only at the regional scale based on mixed-effect models. Soil texture significantly explained temperature sensitivity (Q_{10}) of soil respiration at both scales. Different SOC quality fractions characterized by midDRIFTS played a minor role, whereas extractable organic C related negatively to the respiration Q_{10}. Soil properties controlling soil enzymes (Q_{10}) were scale-specific. We found pH to be the main factor affecting β-glucosidase Q_{10} at the landscape scale. We argue that scale-specificity of variables may depend on homogeneity of study areas and should be considered when exploring SOC dynamics. Our study identified direct and indirect controlling factors affecting soil basal respiration and its temperature sensitivity, providing vital information for SOC dynamics at large scales.

7.1 Introduction

Soil respiration, a primary pathway of soil organic carbon (SOC) loss, plays a significant role in the global C cycle (Chen et al., 2015; Jiang et al., 2015). Globally, 50 – 90 Pg CO_2-C per year are emitted from soils into the atmosphere and it has been suggested that ongoing global warming will increase this flux (Del Grosso et al., 2005; Bond-Lamberty and Thomson, 2010; Subke and Bahn, 2010). Recent studies have estimated global SOC stocks of 510 to 3040 Pg C, a six-fold variation using different models (Todd-Brown et al., 2013), resulting in uncertainty about the response of soil C to changing climate. This demonstrates the need for a better mechanistic understanding of SOC decomposition at large scales (Todd-Brown et al., 2013; Hararuk et al., 2015).

SOC dynamics are controlled by factors like climate, landscape position and biotic properties as well as their complex interactions (Burke et al., 1989; Luo et al., 2017b). For example in regions with similar mineralogy, clay content is usually highly related to SOC stabilization (Burke et al., 1989). Doetterl et al. (2013) demonstrated, however, that understanding spatially variable SOC stocks requires the consideration of multiple environmental processes. Therefore, landscapes differing in the above mentioned soil forming factors might show specific controls of climatic and soil physicochemical and biotic properties on SOC dynamics that demand investigation. Although soil respiration is the primary process by which C is released from the soil, little information about its regulation at the regional scale is available. In the few studies which have focused on the main factors influencing soil respiration, the use of a broad range of explanatory variables such as SOC, soil bulk density and texture, total nitrogen (TN), pH, and C:N ratio have thus far not provided a clear picture at landscape and regional scales (Friedel et al., 2006; Chen et al., 2010; He et al., 2015).

Soil temperature plays an important role in SOC dynamics with usually negative association to SOC density, observed even at large scales (Wang et al., 2014). Since microbial processes largely control the decomposition and stabilization of SOC, the temperature sensitivity of microbial respiration is likely to have a strong influence on the response of SOC content to global warming (Meier et al., 2010; Wieder et al., 2015). On the regional scale, temperature sensitivity of SOC decomposition may be controlled by site specific soil properties such as substrate quality and quantity, as well as other physicochemical soil properties including pH, soil moisture, diffusion

limitation, and soil texture (Davidson and Janssens, 2006; Lützow and Kögel-Knabner, 2009b; Suseela et al., 2012; Yu et al., 2015). Stabilization of available substrate by physical and chemical processes, e.g. isolation within soil aggregates and adsorption onto mineral surfaces, along with its spatial distribution, may also restrict a SOC decomposition response to temperature (Davidson and Janssens, 2006; Poll et al., 2006; Kemmitt et al., 2008; Lützow and Kögel-Knabner, 2009b). Chemical recalcitrance of SOC is also one of the major constraints on the temperature response of decomposition, although contradictory results have been reported (Fang et al., 2005; Knorr et al., 2005; Conen et al., 2006; Fierer et al., 2006; Benbi et al., 2014; Xu et al., 2014). Characterizing SOC quality is, therefore, a promising tool for explaining regional variation in the temperature response of soil respiration.

The quality of SOC has been characterized by a variety of techniques (Lützow et al., 2007). Diffuse Reflectance Fourier Transform mid-infrared Spectroscopy (midDRIFTS) is a high throughput method that has been successfully applied to characterize SOC fractions (Demyan et al., 2013; Kunlanit et al., 2014) making this technique useful for studies at large scales with many samples. Many studies have shown mid-infrared spectral changes both in applied residues and bulk soil during incubation (Spaccini et al., 2001; Calderón et al., 2011; Kunlanit et al., 2014). Studies have also shown differing functional group contributions between light and heavy soil organic matter fractions (Demyan et al., 2012). However, as with other analytical methods, midDRIFTS might have its limits such as overlapping peaks resulting from more than one vibrational functional group and the fact that the spectrum is a result of vibrations of both mineral and organic components (Demyan et al., 2012).

Microbially produced enzymes mediate the rate-limiting step of SOC depolymerization (Kandeler et al., 2006; Min et al., 2014) and a considerable proportion of heterotrophic respiration is controlled by the enzyme activities (Dungait et al., 2012; Ali et al., 2015). To improve our understanding of the temperature sensitivity of soil respiration, it is therefore important to investigate the factors controlling the temperature response of enzyme activities. High temperatures accelerate microbial decomposition of SOC by increasing activities of soil enzymes (Wallenstein et al., 2011), while quantity of organic matter may control or mask the temperature effect on enzyme activities. For example, German et al. (2011) found as much as a 50 % decrease in starch mineralization when its relative contribution was below ca. 10 % of the total SOC due to a reduction

in starch-degrading enzymes. Trasar-Cepeda et al. (2007) found increasing activation energies in soils with increasing amounts of SOC; however, this effect was enzyme dependent. Also changes in microbial community composition may lead to a different spectrum of enzymes and isoenzymes which in turn might have different responses to temperature, thus affecting the observed enzyme temperature sensitivities from C substrates (Davidson and Janssens, 2006; Ali et al., 2015).

In the present study, we sampled soils from two landscapes differing in climatic and edaphic conditions and investigated the data at two scales: landscape scale (representing the two individual areas; the Kraichgau and the Swabian Alb, each around 27 km^2) and regional scale (representing pooled data of both areas, situated approximately 95 km apart from each other). The objective was to investigate the specificity of the factors explaining SOC decomposition dynamics to landscapes differing in their climatic, physicochemical, and biotic properties. We hypothesized that the quality of SOC, as characterized by midDRIFTS, would be a primary factor in explaining basal respiration and its temperature sensitivity due to its respective lability and relevance for microbially-mediated decomposition (soil respiration and enzyme activities). We related landscape / regional variation in basal soil respiration, its short-term temperature sensitivity, and the temperature responses of two soil enzymes degrading SOC of varying complexities (β-glucosidase and xylanase), to a set of biotic, physical and chemical soil properties.

7.2 Materials and methods

Study areas and soil sampling

The two areas of the present study are located in southwest Germany and are part of the integrated research project "Agricultural Landscapes under Global Climate Change – Processes and Feedbacks on Regional Scale" (http://klimawandel.uni-hohenheim.de/) of the German Research Foundation (DFG). The first area lies in the central Swabian Alb (500 – 850 m a.s.l,) characterized by extensively used grass- and croplands with a cool and humid climate (mean annual temperature of 7.0 °C, precipitation 800 – 1000 mm). Soils in this area developed mainly from Jurassic limestone into shallow and stony Leptosols (WRB, 2007). The second is the Kraichgau area (100 – 400 m a.s.l), which is largely covered with loess and is a fertile and intensively cropped hilly area. Soils in the Kraichgau developed mainly into Regosols and Luvisols (WRB, 2007). In comparison to the Swabian Alb, the Kraichgau is characterized by a warmer and drier climate (mean annual temperature of 9.3 °C, precipitation 720 – 830 mm). Average total nitrogen fertilization (between 2010 and 2012) was 227 kg N ha^{-1} in the Kraichgau (170 – 274 kg N ha^{-1}) and 216 kg N ha^{-1} in the Swabian Alb (180 – 244 kg N ha^{-1}). In 2009, soil moisture networks within 27 km^2 domains were installed on agricultural fields in each landscape and consisted of 20 sites in the Swabian Alb and 21 in the Kraichgau (Fig. S7.1). At each site, soil temperature, moisture and precipitation data are continuously recorded. Soil type at each individual site belongs to the main soil types of that landscape. Common crops grown in both landscapes included Rapeseed (*Brassica napus*), Mustard (*Sinapis arvensis*), Barley (*Hordeum vulgare*), Spelt (*Triticum spelta*), Wheat (*Triticum aestivum*) and Pea (*Pisum sativum*), whereas the Swabian Alb had in addition to above mentioned crops also Maize (*Zea mays*), Oats (*Avena*) and different species of clover (*Trifolium*). For a detailed description of the moisture networks, we refer to Poltoradnev et al. (2015). Soil samples from these 41 soil moisture network sites were collected between 17 April and 9 May, 2013. Four soil cores (Ø 2.5 cm) to 30 cm depth were taken from each site, and mixed thoroughly to get one composite sample per site. Samples were kept in cooling boxes, transferred to the laboratory, sieved through a <2 mm sieve and stored at –24 °C until further analysis. Studies have shown no considerable effects of freezing soils to –20 ± 2 °C on soil properties like microbial biomass and basal soil respiration (Stenberg et al., 1998).

SOC characterization

SOC was characterized by Diffuse Reflectance Fourier Transform mid-Infrared Spectroscopy (midDRIFTS). With this technique, organic and inorganic functional groups are characterized by the reflectance of their characteristic bending and stretching vibrations in the mid-infrared range. Soil samples were ball milled and dried overnight at 32 °C before measurement. Soil spectra were scanned on a Tensor-27 Fourier transform spectrometer (Bruker Optik GmbH, Ettlingen, Germany) equipped with a liquid N cooled mid-band mercury-cadmium-telluride detector and potassium bromide beam splitter. A Praying Mantis diffuse reflectance chamber (Harrick Scientific Products, New York, USA) purged with dry air (200 liters $hour^{-1}$ flow rate) using a compressor (Jun-Air International, Nørresundby, Denmark) was mounted to the spectrometer system. Sixteen individual scans at a resolution of 4 cm^{-1} were combined to record the spectra in the mid-infrared range (4000 to 400 cm^{-1}). Each sample was measured three times to gain a representative spectrum. Deeper insights into the methodology used are given in Demyan et al. (2012). Peaks at 2930, 1620, 1530 and 1159 cm^{-1}, integrated with a local baseline for correction, were selected as indicators of organic functional groups representing a range of stabilities within SOM (Demyan et al., 2012). The peak at 2930 cm^{-1} was assigned to C-H vibrations (Stevenson, 1994), the peak at 1620 cm^{-1} to aromatic C=C and/or asymmetric $–COO^-$ stretching (Baes and Bloom, 1989), the peak at 1530 cm^{-1} to aromatic C=C stretching, and the peak at 1159 cm^{-1} to C–O of both polyalcoholic and ether groups (Spaccini and Piccolo, 2007). Relative peak area, with respect to the sum of the 4 peaks (e.g. denoted as r_2930 representing relative peak area at 2930 cm^{-1}), was taken as indicator for shifts in the chemical composition of SOC, i.e. in the quality of SOC. The limits of the individual peaks are given in Fig. 7.1. The peak at 2930 cm^{-1} was corrected for carbonate interference as calcite and dolomite have overlapping peaks with aliphatic vibrations (Mirzaeitalarposhti et al., 2016).

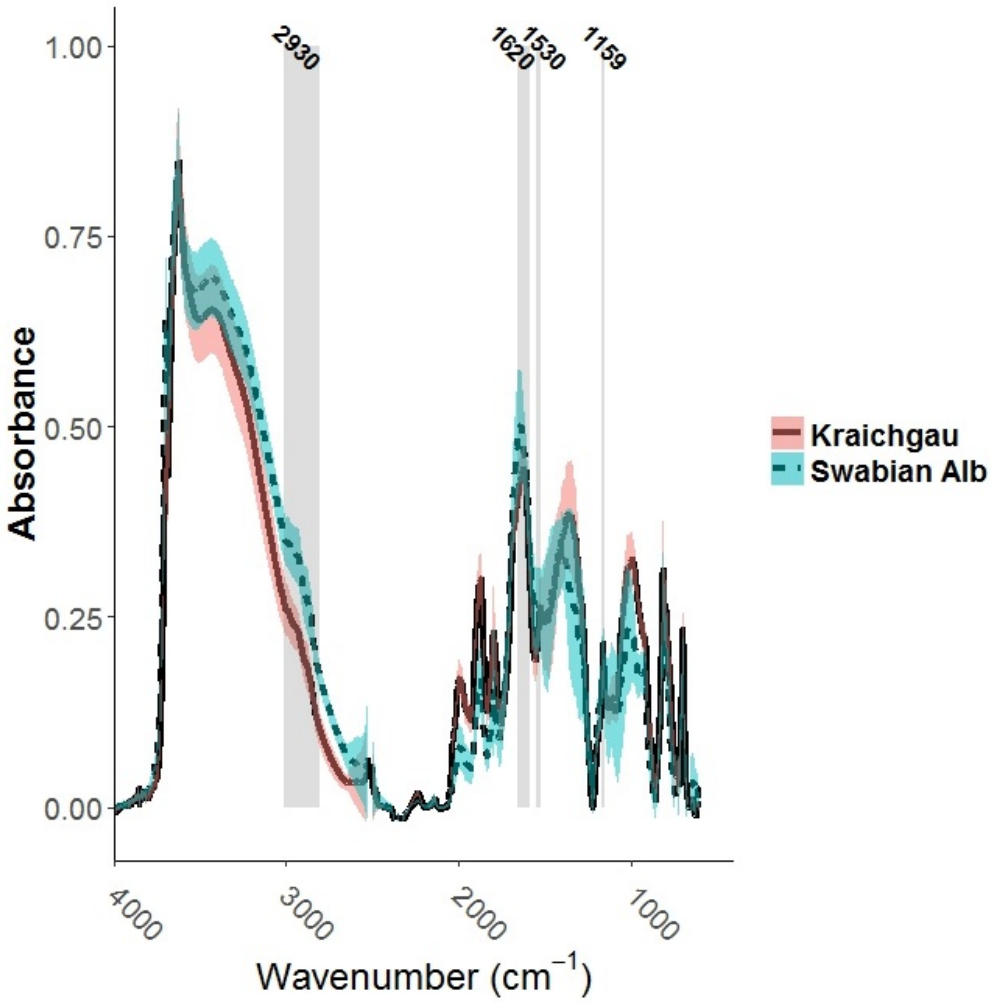

Fig. 7.1 Baseline corrected midDRIFTS spectra from the bulk soil of the Kraichgau (n = 21) and the Swabian Alb (n = 20). The lines (solid and dotted) are the average of all spectra from the respective landscape with the error envelope ± one standard deviation. The grey shaded bars show the integrated regions used for further analysis.

Soil biotic properties

Chloroform fumigation-extraction was used to determine microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) content (Vance et al., 1987). Briefly, 10 g thawed, field moist soil from each sample was fumigated for at least 24 h under alcohol–free chloroform fumes. One non-fumigated sample from each was taken as the control. Extraction of fumigated and control samples was done by adding 40 ml of 0.5 M K_2SO_4, followed by shaking (30 min at 250 rev min^{-1}) and centrifugation (30 min at 4422 x g). Organic C in supernatant was measured with a DOC/TN analyzer (Multi N/C 2100S, Analytik Jena, Germany). Organic C of control samples was subtracted from fumigated

samples and converted into C_{mic} and N_{mic} using the k_{ec} factor 0.45 (Joergensen, 1996) and k_{en} factor 0.54 (Joergensen and Mueller, 1996), respectively. Extractable organic carbon (EOC) and nitrogen (EN) were calculated from the control samples.

Soil fungal biomass was assessed by measuring ergosterol content as a biomarker for fungi following a modified method of Djajakirana et al. (1996). Briefly, 1 g thawed field moist soil was extracted with 50 ml ethanol by shaking at 250 rev min^{-1} on a horizontal shaker followed by centrifugation at 4422 x g for 30 minutes. Twenty ml supernatant solution was dried at 50 °C in a vacuum rotary evaporator (Martin Christ, RVC 2-25, Osterode am Harz, Germany), dissolved in 1 ml methanol, then transferred to 2 ml brown HPLC (High Performance Liquid Chromatography) vials through a 0.45 µm cellulose-acetate filter (Sartorius Stedim Biotech GmbH, Germany). Extracted ergosterol content was measured with HPLC (150 x 3 mm AquaPerfect C18, 3 µm column, flow rate 0.5 ml min^{-1}, detection wavelength 282 nm) using pure methanol as the mobile phase. A standard curve was obtained by using different dilutions of pure ergosterol (Sigma-Aldrich, St. Louis, USA) with final concentrations of 0.1, 0.2, 0.5, 1, 2, and 5 µg ergosterol ml^{-1}.

Soil physicochemical properties

Total carbon (TC) and total nitrogen (TN) were measured with the elemental analyzer MACRO (Elementar Analysensysteme GmbH, Hanau, Germany). Carbonate content was determined using the Scheibler method (Schlichting et al., 1995); treating approximately 1 g soil with HCl for volumetric determination of evolved CO_2. Soil organic C was calculated by subtracting carbonate from TC. pH was determined from 2 g soil suspended in 5 ml of 0.01 M $CaCl_2$ solution using a glass electrode pH meter. Mineral nitrogen (N_{min}), as the sum of ammonium (NH_4^+) and nitrate (NO_3^-), was measured from non-fumigated control extracts of the chloroform-fumigation analysis (see above), using an AutoAnalyzer 3 (Bran & Luebbe, Norderstedt, Germany). Soil texture was measured from individual samples as explained by Poltoradnev et al. (2015).

Temperature sensitivity of soil respiration and enzymes

Temperature sensitivity of SOC decomposition was assessed by measurement of microbial respiration rates via an automated respirometer based on electrolytic oxygen (O_2) micro-compensation (Scheu, 1992). Moist soil (60 % WHC) equivalent to 4 g dry soil was incubated at

four different temperatures; 10, 17, 24, and 31 °C. Incubation at each temperature step lasted for > 72 h (O_2 consumption reached basal rates after 48 h in a preliminary experiment). The following exponential function (Eq. (7.1)) was applied to the average basal respiration rate between 49–72 h to calculate the Q_{10} (denoted by RQ_{10} hereafter) values (Eq. (7.2)) of SOC decomposition:

$$k(T) = k_0 \, e^{aT} \quad (7.1)$$

$$Q_{10} = e^{10 \times a} \quad (7.2)$$

Where T is the incubation temperature (°C), k_0 is the intercept with y-axis at T = 0 °C and a is the exponential coefficient.

Soil enzyme responses to changing soil temperature were assessed by conducting enzyme assays at five different temperature steps (β-glucosidase: 6, 12, 18, 24 and 30 °C; xylanase: 6, 17, 28, 39 and 50 °C). Activities of β-glucosidase and xylanase measured at 30 and 50 °C respectively, were considered as their potential activities (Marx et al., 2001; Ali et al., 2015). The enzymes β-glucosidase and xylanase target organic matter, and refer to the labile and relatively complex C pools, respectively (Schinner and Mersi, 1990; Acosta-Martínez and Tabatabai, 2000). Temperature sensitivity of β-glucosidase activity was measured in 1 g soil using 1 mM substrate concentration (4-methylumbelliferyl-b-D-glucopyranoside), whereas for the xylanase activity measurement, 5 g soil were incubated with 15 ml of 1.2 % xylan solution. Both enzyme activities were measured using 96-well microplates on a micro plate reader. Assay specific steps for measuring temperature sensitivities of individual enzymes are described in Ali et al. (2015). Exponential functions (Eq. (7.1)) were fitted to measured enzyme activities at different temperatures to determine exponential coefficients, which were further used to calculate the Q_{10} values (Eq. (7.2)) for individual enzymes.

Extracting factors affecting Q_{10} of soil enzymes and respiration

A two-step methodology was used to simplify the data analysis procedure; step one included parameter selection through random forests (RF) regression analysis, and in step two the selected parameters from RF regression analysis were used in linear mixed-effect models. In step one, a machine-learning tree ensemble method, RF (randomForest function of the randomForest package

in R version 3.3.2) (Liaw and Wiener, 2002), was used for regression analysis with basal respiration, its Q_{10} and the Q_{10} values of soil enzymes as response or dependent variables, separately, and the other measured soil properties as independent variables using the R language for statistical computation (R Core Team, 2017). RF is a widely used method to find variables relevant to response variable for interpretation purpose or to reduce the number of variables for further analysis. The process involved ranking explanatory variables (500 regression trees; where in a grown tree, each node is split using the best predictor variable among a subset of randomly chosen at that node) using variable importance (VI) scores. When two highly correlated variables were selected in a single model, the variable with the lower rank was removed (ranking based on Person's correlation $\geq$ 0.90) to avoid collinearity, then new trees were calculated. Variable importance scores were averaged over the models to get a final importance value for each variable.

To isolate the most important factors explaining the variability of Q_{10} values, from the tree ensemble model output a relative VI threshold was set as described by Hobley et al. (2015). Variables with VI higher than the set threshold were further used to inform linear mixed-effect models (lme function of the nlme package) (Pinheiro et al., 2015). Predictive variables were taken as fixed effects and a constant independent variable with a random intercept. Different autocorrelation structures were incorporated into the mixed-effect models with respect to the coordinates of the soil sampling points in each area (compound symmetry structure corresponding to uniform correlation, or an exponential spatial correlation structure).

Within the linear mixed-effect models, a sequential variable-drop-strategy was followed for model simplification to get the final model output. To achieve this, variables with the lowest significance (based on the p-value) were dropped and models were re-run until all the variables retained were significant. The final model was selected based on the Akaike information criterion (AIC). Models with the lowest AIC value were considered best resulting in some models with non-significant variables.

Since linear mixed-effect models have no well-established r^2 statistics, fitted lme model performance was assessed by calculating likelihood-ratio-based r^2, an r^2-like statistic, as described by Sun et al. (2010). Calculating such a r^2-like statistics allows comparing different models with each other and helps to find out associated variance-covariance structures of different fixed effects

within mixed-effect models (Sun et al., 2010). We used r^2 of the final lme model to extract the relative importance of each significant variable in explaining the observed variation of each response factor by dropping them one at a time and observing changes in the model r^2.

The entire variable selection procedure explained above was also applied to soil respiration measured at 31 °C in order to find the variables that could be used as indicators of soil respiration efflux at the landscape and the regional scales. Soil respiration data were normalized with respect to SOC content at each site as were the microbial properties (C_{mic}, ergosterol and enzyme potential activities).

7.3 Results

Biotic and abiotic site properties

The relative contributions of organic C quality indices areas characterized by midDRIFTS, averaged over the sampling sites, differed significantly ($P < 0.001$) between the Kraichgau and the Swabian Alb except for the peak area at 1159 cm^{-1} (Fig. 7.1 & Table S7.1). The peak at 1620 cm^{-1} contributed most to the total relative area (i.e. highest contribution to the sum of four selected peaks), followed by the peaks at 2930, 1159 and 1530 cm^{-1}. In the Swabian Alb, abundance of peak at 2930 cm^{-1}, representing the aliphatic C-H stretching, was twice that of Kraichgau (Table 7.1). SOC, carbonate, TN, EOC, EN and N_{min} (NH_4^+, NO_3^-) were also higher in the Swabian Alb than in the Kraichgau. There was considerable variation in all soil properties within and between the two landscapes. Potential xylanase activities related positively and C:N related negatively to basal respiration at both landscape and regional scales, however, their relationship was significant ($P < 0.05$) only at the regional scale. Other properties that showed significant ($P < 0.05$) correlations to basal respiration at the regional scale included SOC, TN, EN, NO_3^- and soil texture (Table S7.2). The RQ_{10} and Q_{10} of β-glucosidase and xylanase activities exhibited different but non-significant correlations to the explanatory variables at both landscape and regional scales (Table S7.2).

Table 7.1 SOC chemical quality indices (represented by stretching and vibrations of different C groups at peaks 2930, 1620, 1530 and 1159 cm^{-1}) as well as measured soil physical, chemical, and biotic properties in the Kraichgau and the Swabian Alb. Values represent average, standard deviation, and coefficient of variation of the sites from the Kraichgau (n = 21) and the Swabian Alb (n = 19).

	Kraichgau			Swabian Alb		
Variables	**Average**	**Std.dev**	**CV (%)**	**Average**	**Std.dev**	**CV (%)**
r_2930 (cm^{-1})	20.06	10.73	53	41.98	15.73	37
r_1620 (cm^{-1})	67.56	8.37	12	47.66	12.53	26
r_1530 (cm^{-1})	3.05	1.12	37	1.36	1.08	79
r_1159 (cm^{-1})	9.44	1.79	19	9.01	3.00	33
pH	6.68	0.83	12	6.84	0.72	10
SOC (%)	0.97	0.19	19	2.82	0.80	29
Carbonate-C (%)	0.48	0.79	167	0.83	1.29	156
TN (%)	0.12	0.02	15	0.31	0.08	27
Soil C:N	7.85	1.29	16	8.95	0.63	7
C_{mic} (µg C mg^{-1} SOC)	180.11	48.24	27	174.88	42.70	24
N_{mic} (µg N mg^{-1} SOC)	20.58	7.41	36	22.59	7.07	31
EOC (µg g^{-1} soil)	50.28	8.11	16	80.59	30.10	37
EN (µg g^{-1} soil)	20.59	7.53	37	37.88	12.19	32
NO_3^- (µg g^{-1} soil)	21.63	11.54	53	46.91	16.17	34
NH_4^+ (µg g^{-1} soil)	1.67	0.73	44	2.32	2.18	94
Clay (%)	22.42	4.93	22	52.34	13.41	26
Sand (%)	4.36	2.83	65	3.70	2.06	56
Silt (%)	73.22	5.26	7	45.92	12.87	28
Ergosterol (µg mg^{-1} SOC)	1.40	0.48	34	0.94	0.26	27

EOC = extractable organic C

Temperature sensitivity of soil respiration and enzyme activities

Basal soil respiration (at 31 °C measured as O_2 consumption) ranged from 1.47 – 2.86 and 0.74 – 2.42 µg O_2 mg^{-1} SOC h^{-1} in the Kraichgau and the Swabian Alb, respectively (Fig. 7.2). Soil respiration increased with increasing temperature in the short-term incubation experiments, with the lowest flux recorded at 10 °C and highest at 31 °C (Fig. S7.2). At all temperature steps, we recorded 54 – 91 % higher relative soil respiration from the Kraichgau samples than from the Swabian Alb. Temperature sensitivity of soil respiration was not significantly different between

the two study areas (Fig. 7.3). We recorded similar variations in RQ_{10} values in the Kraichgau and the Swabian Alb (coefficients of variation 14 and 13 %, respectively) ranging from 1.37 to 2.67 in the Kraichgau (median 1.95) and from 1.47 to 2.60 in the Swabian Alb (median 1.88). At the regional scale, the RQ_{10} coefficient of variation was 13 % (median 1.90).

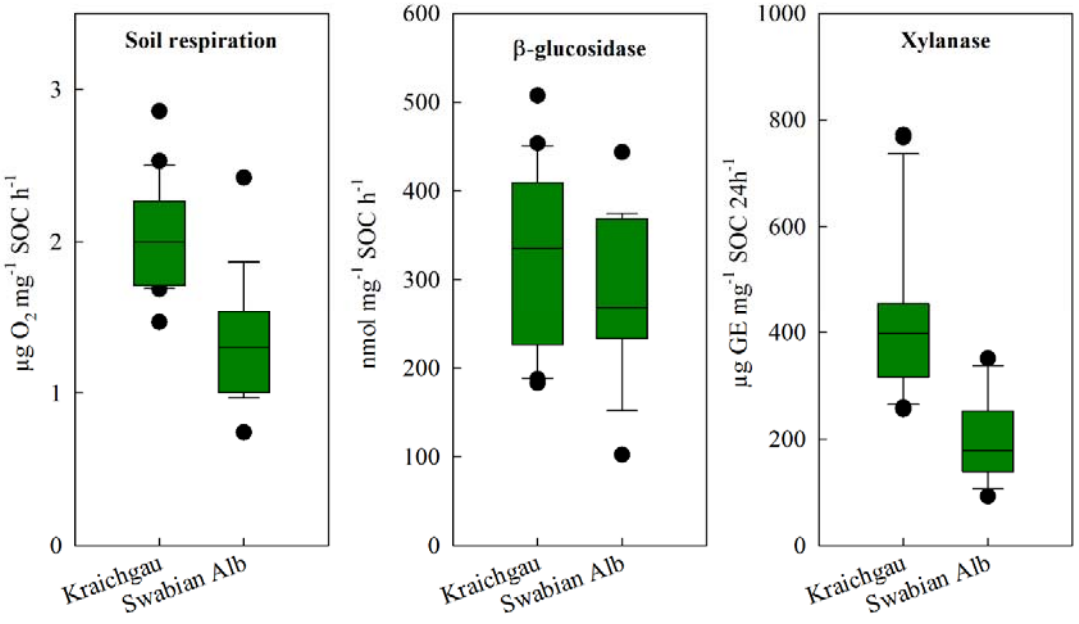

Fig. 7.2 Basal soil respiration (measured at 31 °C) and potential activities of β-glucosidase (measured at 30 °C) and xylanase (measured at 50 °C) for the Kraichgau (n = 21) and the Swabian Alb (n = 19).

Enzyme potential activities in the Kraichgau ranged from 183 – 507 nmol mg^{-1} SOC h^{-1} for β-glucosidase and 256 – 772 µg GE mg^{-1} SOC 24 h^{-1} for xylanase (Fig. 7.2). In the Swabian Alb, potential enzyme activities ranged from 102 – 443 nmol mg^{-1} SOC h^{-1} and 92 – 351 µg GE mg^{-1} SOC 24 h^{-1} for β-glucosidase and xylanase, respectively. Over the incubation temperature range, β-glucosidase activities were 10 – 25 % and xylanase activities 63 – 150 % higher in the Kraichgau than in the Swabian Alb (Fig. S7.3). The two landscapes did not differ significantly from each other with respect to the Q_{10} of soil enzymes (Fig. 7.3). β-glucosidase Q_{10} ranged from 1.42 to 2.18 (median 1.82) in the Kraichgau and 1.51 to 1.94 (median 1.76) in the Swabian Alb with greater variability in the Kraichgau (CV 11 %) than the Swabian Alb (CV 6 %). Xylanase Q_{10} ranged from

1.34 – 2.80 (median 1.65, CV 19 %) in the Kraichgau and 1.31 – 1.87 (median 1.59, CV 10 %) in the Swabian Alb. At the regional scale, β-glucosidase Q_{10} values were slightly higher (median 1.77, CV 9 %) than were those of xylanase (median 1.61, CV 16 %).

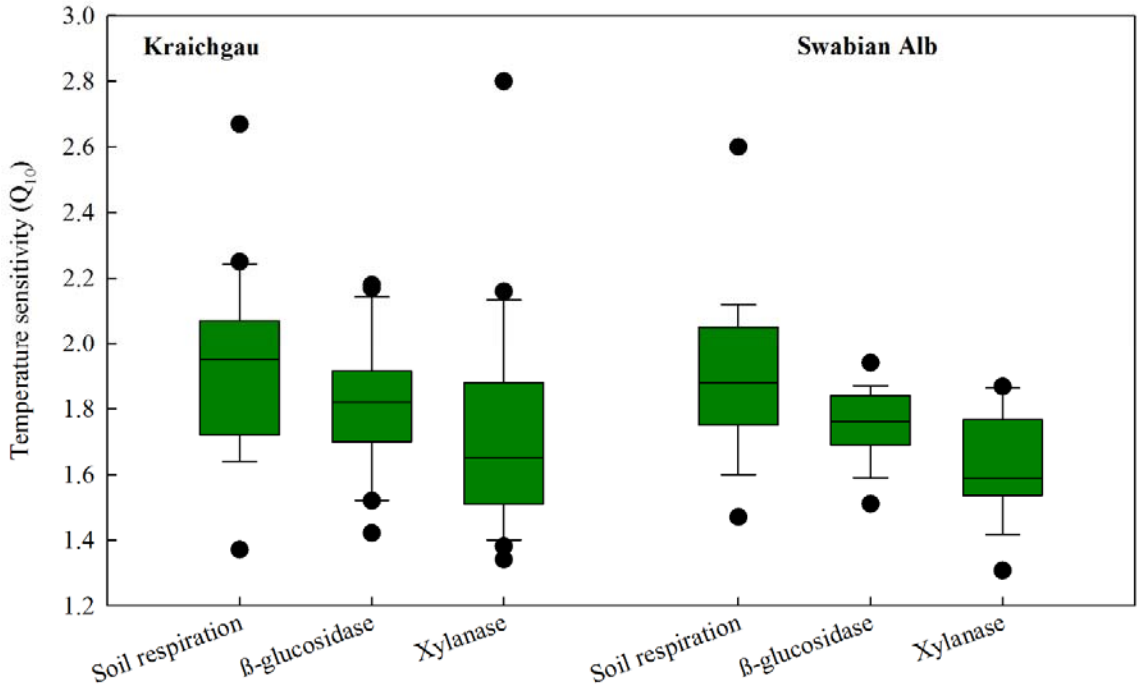

Fig. 7.3 Temperature sensitivities of soil respiration and the two enzymes (β-glucosidase and xylanase) for the Kraichgau (n = 21) and the Swabian Alb (n = 19).

Variable selection by tree ensemble models

At the landscape scale

The variable selection procedure by tree ensemble models extracted C:N, pH and soil texture as common explanatory variables explaining soil basal respiration in both landscapes; soil organic C quantity was most important in the Kraichgau and soil carbonate in the Swabian Alb (Fig. S7.4 a,b). Explanatory variables for the Q_{10} of soil respiration were clay, ranked highest in importance in the Kraichgau, and ammonium in the Swabian Alb. Other variables which ranked important in both landscapes included EOC, TN, pH, r_1159 and sand (Fig. S7.4 c,b). To explain the Q_{10} of β-glucosidase, pH and SOC were the two most important variables in both landscapes (Fig. S7.5 a,b),

whereas ammonium and potential xylanase activities ranked highest in explaining the Q_{10} of xylanase (Fig. S7.5 c,d).

At the regional scale

Tree ensemble models selected SOC, soil texture, C_{mic}, potential xylanase activities and organic C quality indices as important variables in explaining basal respiration and its temperature sensitivity. SOC ranked highest in importance in explaining RQ_{10}; however, it ranked second in explaining basal respiration after C:N (Fig. S7.6). Soil texture, organic C quality indices, pH, carbonate, potential β-glucosidase activities, and fungal biomass were important explanatory variables common to both β-glucosidase and xylanase with respect to temperature sensitivities (Fig. S7.6).

Factors affecting Q_{10} variation in soil respiration and enzyme activities

At the landscape scale

Linear mixed-effect models performed almost equally in selecting factors to explain basal soil respiration measured at 31 °C in both areas (58 % explained variance in the Kraichgau and 57 % in the Swabian Alb). SOC quantity was significant in explaining basal respiration in the Kraichgau, whereas C_{mic} was significant in the Swabian Alb (Table 7.2). With respect to temperature sensitivity of soil respiration, mixed-effect models performed better in the Swabian Alb, explaining 43 % of RQ_{10} variance, compared to 39 % variance explained in the Kraichgau. Soil texture (clay content) was the common factor explaining RQ_{10} in both landscapes. In addition to soil texture, pH was significant in explaining RQ_{10} in the Kraichgau, while in the Swabian Alb quantity of EOC was significant (Table 7.2). Soil pH was the most important variable explaining the temperature sensitivity of β-glucosidase activities in both landscapes. In the Kraichgau, in addition to potential β-glucosidase activities, SOC quantity was significant in explaining β-glucosidase Q_{10}, whereas in the Swabian Alb, soil organic C quality (SOC quality indices and C:N) were significant. No measured soil properties were considered significant in explaining xylanase Q_{10} values in the Kraichgau, whereas in the Swabian Alb only potential xylanase activities were significant in explaining the xylanase Q_{10} (Table 7.2).

At the regional scale

Pooling the basal soil respiration data of the two landscapes improved the performance of the mixed-effect model (r^2 = 0.65) and identified soil C:N and biotic properties including C_{mic} and potential xylanase activities as significant explanatory variables (Table 7.2). Pooling soil respiration temperature sensitivity data decreased the performance of the mixed-effect model (r^2 = 0.17) and resulted in C_{mic} and soil texture as significant explanatory variables for RQ_{10} (Table 7.2). Potential β-glucosidase activity remained significant at the regional scale in explaining the observed β-glucosidase activities' Q_{10} values. Although model performance in explaining xylanase temperature sensitivity decreased at the regional scale, r_2930 and clay content were selected as significant indicators (Table 7.2).

Table 7.2 Results of linear mixed-effect models showing explanatory variables for basal soil respiration (31 °C), its temperature sensitivity and Q_{10} of enzyme activities at landscape and regional scales. Arrangement of variables represents significant importance based on reduction in the pseudo r^2-*like* parameter calculated by dropping each significant variable one at a time. Decisions on which non-significant variables in the model to keep was based on the AIC values. Variables in bold are statistically significant ($P < 0.05$). $XylQ_{10}$ and βQ_{10} are the Q_{10} of xylanase and β-glucosidase activities, respectively.

Landscape scale			
Kraichgau		**Swabian Alb**	
	r^2		r^2
Basal soil respiration ~ **SOC** + NH_4^+ + Silt	0.58	Basal soil respiration ~ $\mathbf{C_{mic}}$ + β-glucosidase	0.57
RQ_{10} ~ **Clay** + **pH**	0.39	RQ_{10} ~ **EOC** + **Clay** + r_1159 + NH_4^+	0.43
Q_{10} of enzyme activities			
βQ_{10} ~ **pH** + **β-glucosidase** + Ergosterol + **SOC**	0.45	βQ_{10} ~ **pH** + **C:N** + **r_1530** + **NH_4^+** + **r_1159** + β-glucosidase + Carbonate + SOC	0.90
$XylQ_{10}$ ~ r_1159 + β-glucosidase	0.37	$XylQ_{10}$ ~ **Xylanase**	0.30
Regional Scale (pooled data from Kraichgau and Swabian Alb)			
Basal soil respiration ~ **C:N** + $\mathbf{C_{mic}}$ + **Xylanase** + Nitrate			0.65
RQ_{10} ~ $\mathbf{C_{mic}}$ + **Silt** + Nitrate + r_1159			0.17
Q_{10} of enzyme activities			
βQ_{10} ~ **β-glucosidase** + Ergosterol			0.22
$XylQ_{10}$ ~ **r_2930** + **Clay**			0.19

7.4 Discussion

Given the large variations in predicted SOC estimates, it is important to identify and understand the control mechanisms of SOC decomposition dynamics that may help to make SOC estimates more accurate. Integrating spatially variable Q_{10} values in global soil respiration models have been shown to increase total (40 %) and microbial (25 %) respiration (Zhou et al., 2009). The spatial variability of soil respiration Q_{10} and its control factors have also been measured for different regions and ecosystems with varying properties (Zheng et al., 2009; Liu et al., 2017). In this study, we have investigated the specificity of the factors explaining SOC dynamics (soil respiration, its temperature sensitivity and sensitivity of two enzymes) to two landscapes differing in their climatic and edaphic conditions at two spatial scales: landscape scale (representing the two individual areas) and regional scale (representing pooled data of both landscapes).

Basal soil respiration

Basal soil respiration data is presented as specific respiration (i.e. g^{-1} SOC) to identify underlying drivers, which are independent of SOC content. One important aspect to mention here is the control of soil moisture over microbial C dynamics; by soil moisture strongly affecting soil respiration (Chang et al., 2014). We, however, measured basal soil respiration and its temperature sensitivity at optimal moisture content (60 % WHC) and enzyme activities in a soil slurry, which makes the control of soil moisture irrelevant. In our study, microbial biomass (C_{mic}) was the only factor significantly explaining basal soil respiration in the Swabian Alb (Table 7.2). An association of soil respiration with the C_{mic} pool has been found in previous studies (Wang et al., 2003; Fraser et al., 2016) and also been implemented in many decomposition models (Zhang et al., 2014; Wieder et al., 2015). However, this relationship is complex and substrate supply to microbial or enzymatic sites may be limited by other physio-chemical properties, for instance substrate stabilization in micro-aggregates and/or its chemical stabilization at the surface of clay minerals (Birge et al., 2015). Care must also be taken in directly associating microbial biomass with the respiration flux since as temperature increases microbial C use efficiency decreases and more CO_2 is respired from the same microbial pool (Manzoni et al., 2012b). The fact that C_{mic} was the only significant explanatory variable for basal soil respiration in the Swabian Alb may indicate that C_{mic} integrates

the effects of all habitat conditions, whereas each habitat condition alone may have only a small effect.

Quantity of SOC significantly explained basal soil respiration in the Kraichgau but not in the Swabian Alb, where organic C was almost three times higher than in the Kraichgau (Table 7.1). Normalization of the respiration data to SOC content, as in our study, neutralizes the overwhelming direct effect of SOC quantity on respiration and implies that indirect effects of SOC content connected to other factors are important controls on soil respiration. The warmer weather conditions of the Kraichgau compared to the Swabian Alb are usually preferable for microbial activity, suggesting that labile C sources are more efficiently degraded. This is supported by the relative peak area at 2930 cm^{-1}, used as the indicator for a more labile C fraction, which was much lower in the Kraichgau than the Swabian Alb (Table 7.1). We argue, therefore, that variability in SOC content in the Kraichgau was mainly related to differences in the labile C fractions and that this variability strongly influenced soil respiration. In the Kraichgau, therefore, SOC quantity plays an important role due to low levels of substrate, whereas in areas with higher SOC content, e.g. the Swabian Alb, other physical and biological properties integrate the substrate effect (e.g. C_{mic}, see above). Other studies have shown relative increases in the proportion of labile C with overall increasing SOC. Wang et al. (2003) concluded that under optimal temperature and moisture, soil respiration is generally limited by substrate available for biological activity.

At the regional scale, C_{mic} remained a significant variable, together with soil C:N ratio and potential xylanase activities, in explaining basal soil respiration (Table 7.2). Bradford et al. (2017) identified microbial biomass as a strong regulator of litter decomposition rates at the regional scale. A study by Colman and Schimel (2013) also showed a direct control of microbial biomass over soil respiration even at a larger scale than considered in our study. Climate and geology are quite homogeneous within the landscape but differ between both landscapes. Still we saw considerable variation of measured soil properties within each landscape, but this variation was much larger between the landscapes (Table 7.1, Figure S7.7). We suggest, therefore, that the selection of factors explaining soil respiration at the landscape or regional scale depends on homogeneity of measured variables within the target area. Based on our results, one could select two groups of factors: 1) properties that are important as explanatory independent of the spatial scale, e.g. C_{mic} in our study,

and 2) properties that become explanatory as the range of their measured values increase when the target area is extended across different landscapes.

Soil C:N ratio belonged to the second group of factors in our study, with a negative relationship of C:N ratio to basal soil respiration (Table 7.2). The effect of C:N ratio on soil basal respiration can be interpreted from the perspective of substrate quality. Low C:N ratios are usually associated with high quality substrates, which are preferentially degraded by soil microorganisms (Davidson and Janssens, 2006; Cong et al., 2015; He and Yu, 2015; Wang et al., 2015). Taking C:N ratio as a quality index would, therefore, result in low specific respiration with high C:N ratio. In contrast, a high C:N ratio may reduce microbial carbon-use efficiency (CUE) (Manzoni et al., 2012b; Alberti et al., 2014). Consequently, increasing nutrient availability, e.g. through N fertilization of agricultural systems, may increase microbial CUE, leading to reduced respiration (Manzoni et al., 2012b). Nevertheless, mechanisms controlling soil C dynamics through nutrient limitation, particularly N, are still poorly understood (Alberti et al., 2014) and substrate stoichiometry might have an important, yet not fully understood influence on SOC decomposition.

Temperature sensitivity of soil respiration

For understanding large scale drivers of soil C dynamics, not only factors driving soil respiration but also factors driving its temperature sensitivity are important. The site-specific properties are assumed to be in equilibrium at our study sites as no land use change has occurred and therefore, microbial communities are adapted to their local environment. Temperature sensitivity of microbial respiration (RQ_{10}) observed in both landscapes cover the range previously observed by Poll et al. (2013) at the plot scale for an agricultural soil in Germany. Area-specific trends were not visible for RQ_{10} values in this study, which may indicate that similar factors are responsible for within-area variation of Q_{10} values in both, the Kraichgau and the Swabian Alb.

Soil texture was significant in explaining RQ_{10} in both landscapes of our study (Table 7.2). Previous studies have focused on the importance of soil texture from a physico-chemical perspective, with a well-established relationship between clay and SOM (Six et al., 2002; Leifeld and Kögel-Knabner, 2005). However, a microbial C processing perspective is required in order to improve our understanding of the factors that control SOC dynamics since microbes are largely responsible for SOC decomposition. Since we normalized respiration data to SOC content, we consider, based

on our results for basal soil respiration, that the effect of texture on soil respiration via its SOC stabilizing function is less important. Nevertheless, it is important to mention here that the temperature sensitivity of SOC decomposition may depend on the availability of substrate to soil microorganisms/enzymes which in turn is controlled by soil texture through, for example, physical protection, sorption to mineral surfaces or co-precipitation with minerals (Oades, 1988; Davidson and Janssens, 2006; Haddix et al., 2011). Clay may also control the temperature sensitivity of soil respiration through increased soil water holding capacity, soil nutrient availability and changing solution chemistry through pH buffering, leading to greater microbial decomposition of available organic C or directly by interacting with microbes and altering their metabolism (Sollins et al., 1996; Chen et al., 2010). Since aggregate turnover, substrate desorption, and diffusion are strongly affected by texture and increase with increasing temperatures (Conant et al., 2011), spatial heterogeneity in texture may induce differences in the Q_{10} of soil respiration. Basal respiration is measured at a specific temperature and, therefore, this indirect temperature-texture control on RQ_{10} may not apply to basal respiration. In addition, temperature-related decomposition of available substrate by microbial decomposers might also be controlled by substrate quality, which might be regulated by soil texture through mechanisms listed above. In our study, EOC significantly explained the RQ_{10} in the Swabian Alb (Table 7.2). Although there is a considerable debate in the literature, many studies argue that high temperature sensitivities are associated with stable organic C pools, which have complex structures and require high activation energies (E_a), while low temperature sensitivities are associated with labile organic C pools (Fierer et al., 2005; Davidson and Janssens, 2006; Hartley and Ineson, 2008). In the Swabian Alb, we observed a weak but negative correlation between RQ_{10} and EOC. We assume that in our study EOC is mainly driven by the labile SOC fraction which is relatively easily desorbed from the mineral phase and, therefore, it seems to be accessible to the microbes. This was confirmed by a positive correlation of EOC with r_2930 ($R = 0.50$) and a contrasting correlation with r_1620 ($R = –0.51$). These both peaks corresponding to different functional groups (2930 cm^{-1} to aliphatic and 1620 cm^{-1} to aromatic) have been assumed to represent labile and more stable SOC fractions, respectively (Demyan et al., 2012). A similar effect was visible when data from both areas were pooled (r_2930, $R = 0.56$). Our study, at least with SOC quality indices used here, supports, therefore, the idea of labile SOC pool being negatively associated to the Q_{10} of soil respiration even at the landscape and regional scale.

Of the measured chemical properties, pH was the only variable that significantly explained the RQ_{10} in the Kraichgau (Table 7.2). In a study by Craine et al. (2010), 67 % of the observed variance in activation energy of soil organic matter decomposition was explained by pH. This may explain the significant role of pH for RQ_{10} in the Kraichgau, since temperature sensitivity of respiration is directly related to the activation energy of the substrates. In addition, soil pH may affect temperature related SOC decomposition through direct or indirect control on SOC decomposers or SOC availability. Direct pH control could occur by changing bacterial and fungal growth, an effect observed previously in different ecosystems (Bååth and Anderson, 2003; Rousk et al., 2009). An indirect pH control could be through, e.g., alteration of nutrient availability, substrate solubility, and various microbial enzyme activities, which in turn could change microbial resource availability (Acosta-Martínez and Tabatabai, 2000; Bååth and Anderson, 2003; Min et al., 2014).

At the regional scale, soil texture remained a significant factor explaining RQ_{10} together with C_{mic}. However, pH and SOC quantity, significant at the landscape scale, were not selected as important by the mixed-effect models (Table 7.2). Differences in the relevance of environmental factors in explaining RQ_{10} at the landscape and regional scales may indicate that regulation of the temperature response of microbial activity differs slightly at these scales. As discussed for basal respiration, C_{mic} may integrate the effects of all habitat conditions, whereas each habitat condition alone may have only a small effect.

Temperature sensitivity of soil enzyme activities

Roughly half the soil CO_2 flux derives from microbial respiration of SOC, and a significant proportion of this depends on the activity of soil enzymes, which perform the rate-limiting step in SOC decomposition (Wallenstein et al., 2011). Since factors affecting enzyme activities may limit C flux to microorganisms, it is important to understand these factors and the temperature related enzymatic reactions in order to improve predictions of respiratory C losses from soil. However, a challenge remains to determine the controls on the temperature sensitivity of extracellular enzymes directly from current enzyme assay procedures. Generally, in enzyme assays, optimal substrate concentrations are calibrated to the type of targeted enzymes. Therefore, soil indigenous substrate availability may play a very limited role in clarifying observed temperature responses. Other controlling factors, e.g., substrate / enzyme diffusion limitations, are also reduced by performing

assays in a soil slurry (Ali et al., 2015). An additional challenge that makes the link between extracellular enzymes and soil organic C dynamics difficult is their substrate association especially in mineral soils. It might be that most of the organic C associated to minerals is of labile quality and either not accessible by enzymes due to physical barriers and sorption to mineral surfaces or does not require enzymes at all since the investigated enzymes cleave oligomers of very high molecular weights or polymers (Davidson and Janssens, 2006; Colman and Schimel, 2013; Schimel et al., 2017). We argue, therefore, that biotic and abiotic properties investigated in our study influenced temperature responses of measured enzymes only indirectly either by changing the abundances of different isoenzymes with specific temperature sensitivities (Wallenstein et al., 2009; Conant et al., 2011), or by altering their substrate affinity through changes in conformation and sorption to mineral surfaces (Davidson and Janssens, 2006; Min et al., 2014). Furthermore, temperature itself affects the conformation of enzymes (Daniel et al., 2008) and the thermal optima of sorbed enzymes may differ from that of enzymes in solution (Wallenstein and Burns, 2011).

Soil pH was the most significant factor related to the Q_{10} of β-glucosidase activities in both landscapes (Table 7.2). Soil pH affects microbial biomass, its dynamics, and diversity and, therefore, may significantly change enzyme activities (Acosta-Martínez and Tabatabai, 2000). Since different isoenzymes have different pH optima (Turner, 2010; Leifeld and Lützow, 2013), pH may control temperature sensitivity of enzymes through shifts in the composition of enzyme pools. In addition, soil pH also plays an important role in controlling enzyme conformation and its sorption onto the soil matrix (Min et al., 2014), which in turn may influence the temperature response of enzymatic processes. Min et al. (2014) concluded from their study that ecosystems varying in soil pH may have distinct β-glucosidase activity responses to temperature. When data of both study areas were pooled for β-glucosidase Q_{10}, the pH effect disappeared (Table 7.2). Therefore, the direct mechanisms behind pH effects on temperature sensitivities of SOC decomposition need to be further clarified.

Only potential xylanase activity related significantly to the Q_{10} of xylanase activities at the landscape scale (Swabian Alb) whereas at the regional scale, SOC quality and soil texture (clay content) were significant (Table 7.2). Processes by which soil texture may influence the temperature sensitivities of SOC decomposition and enzymes are discussed above. However, the exact mechanism by which substrate availability (r_2930) affects the temperature sensitivity of

xylanase, which is measured under conditions of substrate saturation, remains unclear and needs to be further investigated.

7.5 Conclusions

In conclusion, our study provides vital information about the factors controlling microbial SOC decomposition at two levels of complexity in terms of spatial scale. We argue that homogeneity of the study area with respect to climatic and edaphic properties exerts a strong influence on the relevance of factors explaining soil processes at the investigated scale. Microbial biomass significantly explained SOC-normalized basal respiration at the landscape (Swabian Alb only, covering approximately 27 km^2) and the regional scale (data of both landscapes merged). Soil texture is often discussed as an important control on SOC stabilization. However, in our study it was not an important control on basal soil respiration at any spatial scale, whereas soil texture was an important control on the temperature sensitivity of soil respiration at landscape and regional scales. Controls on the temperature sensitivities of enzymes degrading SOC of varying stabilities showed strong scale-specificity, which needs further investigation due to methodological constraints. The relative importance of direct and indirect controls of SOC dynamics provided by our study using observations from two landscapes differing in climatic and edaphic conditions might have important implications for reliable SOC estimates.

7.6 Acknowledgements

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8 General discussion

Despite the critical role played by soil microorganisms in transforming SOC, including its formation and turnover rates, C modelling studies focus more on soil physical rather than soil microbiological processes as primary controls on soil C dynamics, resulting in great uncertainty about the response of SOC decomposition dynamics to predicted climate change (Balser, 2005; Graham et al., 2014). This bias could be due to limited available information on the highly complex responses of soil microbes to possible climate change factors; improving this knowledge base could advance spatially explicit biogeochemical models (Allison et al., 2010; Waldrop et al., 2017). The main objective of this thesis was, therefore, to investigate the role of soil microorganisms in controlling SOC decomposition at the regional scale, specifically focusing on microbial responses to varying soil temperature, moisture, and C resources differing in quality or availability. This would provide information on the dependence of microbially related SOC decomposition processes on factors driving climate change, dependence that could vary depending upon microbial adaptation strategies. This would in turn help resolve the scale issue by identifying relevant controls on SOC decomposition at different spatial scales. Determination of how soil microbial abundances and functions are influenced by variations in climatic and edaphic properties was made by selecting two study areas: the cool and wet "Swabian Alb" plateau, and the warm and dry "Kraichgau" in southwest Germany. It is important to point out here that the present thesis does not test the sensitivity of soil C models in any way; rather it provides data on the role of microbes with respect to C dynamics in a changing environment, data that can be further utilized for model parameterization or validation purposes.

8.1 Responses of soil microbial abundances and activities to soil temperature and moisture alterations

Temperature is a strong regulator of soil microbial respiration, microbial community abundance and structure, and a strong influence on production and activity of microbial extracellular enzymes, which act as the rate limiting agents of SOC decomposition (Machmuller et al., 2016; Waldrop et al., 2017). Temperature may not only affect soil microbial activity directly, but also indirectly, by modifying other drivers such as soil moisture and substrate availability (Bååth, 2018). Such direct and indirect effects of temperature on microbial respiration (Chapters 5, 6 and 7), microbial enzyme

activities, both potential and *in situ* activities (Chapters 5 and 7), and on microbial community composition and abundance (Chapter 6) were observed in the framework of the present thesis. Soil respiration response to temperature was independent of the spatial scale; increasing temperature induced high soil respiration rates in studies at the plot scale (Chapters 5 and 6) and also at the regional scale (Chapter 7). Maximum soil respiration was observed at highest incubation temperatures in the lab (Figs. 6.1, S6.1; Fig. S7.2) and higher respiration was observed in summer than in winter in the field (Fig. 5.4a,b). This observed control of soil temperature over soil respiration is consistent with other studies (Davidson et al., 1998). Different processes may be responsible for temperature-induced increases in soil respiration, however. For example, given no change in microbial community composition, increasing temperature increases the metabolic activity of soil microbes and the catalyzing efficiency of the extant extracellular enzyme pool (Wallenstein et al., 2011; Classen et al., 2015) leading to more substrate available for microbial utilization, hence increased respiration. This argument is supported by the observed increase in potential enzyme activities with increasing temperatures (Fig. S7.3). Soil warming may also affect microbial carbon use efficiency (CUE), whereby a higher proportion of substrate taken up by microbes is utilized for cell maintenance and less is invested in cell growth, leading to higher respiration rates but low microbial biomass (Steinweg et al., 2008; Frey et al., 2013). This argument is supported by the observed decline in total microbial biomass with increasing temperature that coincided with increasing cumulative respiration (Figs. 6.1, 6.2a, b). Together, these observations suggest that soil warming may enhance the positive feedback of SOC decomposition to climate change by introducing more CO_2 to the atmosphere.

Microbial soil respiration response and its temperature sensitivity may also be decoupled from the temperature sensitivity of enzyme activity, since products catalyzed by enzymatic reactions must go through diffusion limitation before respiration can occur (Wallenstein et al., 2011). Information gained of the temperature sensitivity of SOC pools differing in complexity, using extracellular enzymes as proxies for C quality, were compared at different scales; i.e., plot scale enzyme Q_{10} (Chapter 5) *vs.* landscape to regional scale enzyme Q_{10} (Chapter 7). The range of measured Q_{10} values of β-glucosidase and xylanase activities at the plot scale (Table 5.1) encompassed, to a large extent, the range observed at the landscape scale (Fig. 7.3). That indicated consistency of temperature sensitivities of these enzymes in investigated areas which could be associated with homogeneous geological and climatic properties within each area (Table 7.1). Therefore,

depending upon the homogeneity of the study area with respect to physicochemical and biological factors, temperature sensitivity of soil enzymes at the plot scale might be extrapolated to larger scales, e.g., landscape and regional scales. Regions with considerable variation in physicochemical factors, however, including soil texture, mineralogy, and soil moisture might result in scale dependent temperature responses of microbial enzymes by controlling their activities (Wallenstein et al., 2011).

Soil organic carbon decomposition is potentially one of the largest feedbacks to climate change; therefore, understanding the relative decomposition rates of SOC pools of varying qualities and their respective sensitivity to soil warming at large scales will help improve the accuracy of predicting the fate of SOC in a warmer world (Craine et al., 2010; Davidson et al., 2012). This thesis also investigated the "C quality temperature" hypothesis, which stats that SOC pools that differ in complexity also exhibit different temperature sensitivities, recalcitrant pools being more temperature sensitive than labile pools. The measured Q_{10} values of two enzymes targeting SOC pools of different stabilities confirmed this hypothesis; β-glucosidase activity had, on average, lower Q_{10} values than the Q_{10} of xylanase activities at the plot scale and this effect was consistent in both areas (Table 5.1, Chapter 5). Additionally, extractable organic C (EOC) also associated negatively with the Q_{10} of soil respiration at the landscape scale (Swabian Alb, Table 7.2), (It was assumed that EOC was driven by the labile C fraction due to a positive correlation of EOC with labile C fraction, 2930 cm^{-1} peak from MIRS spectrum; Table S7.2; Chapter 7). A negative relationship of substrate quality with its temperature sensitivity has been observed in previous studies (Liu et al., 2017; Craine et al., 2010; Wang et al., 2018). From these observations and the above-mentioned range of enzyme Q_{10} values, two conclusions could be drawn: 1) the temperature sensitivity of SOC decomposition deviates strongly from a constant assumption of 2 and its variation should be added into C models, and 2) the recalcitrant SOC pool is more sensitive to temperature change than the labile SOC pool. Therefore, in the context of climate change, soil warming may accelerate decomposition of SOC even more strongly by accelerating the relative decomposition rates of recalcitrant C pools which, in most soils, is proportionally much larger than the labile C pool (Davidson and Janssens, 2006). However, a study carried out in the framework of this thesis investigating temperature sensitivity responses of extracellular enzymes at the regional scale found different patterns of enzyme Q_{10}; average xylanase Q_{10} values were slightly but significantly lower than Q_{10} values of β-glucosidase (Fig. 7.3; Chapter 7). This observation does

not necessarily mean that the "C quality temperature" hypothesis may not apply at larger spatial scales, rather it hints towards other mechanisms that may confound the temperature-sensitive responses of extracellular enzyme activities. For example, soil moisture limitation effects on enzyme Q_{10} at the temporal scale (Chapter 5) might not be depicted in measured Q_{10} at the spatial scale (Chapter 7) or *vice versa;* the effect of a spatially variable factor such as clay content might not be observed at the temporal scale (Davidson, 1995; Martin and Bolstad, 2009). Furthermore, relative abundances of microbial taxa have been shown to co-vary with soil properties at the spatial scale (Philippot et al., 2009) and different taxa were also specifically influenced by temperature variations under availability of substrates of varying complexity (Fig. 6.5, Fig. S6.6). Different isoenzymes, enzymes with similar function but different structures, produced by these spatially variable taxa with different temperature sensitivities (Wallenstein and Weintraub, 2008; Wallenstein et al., 2009) may also explain the observed enzyme Q_{10} results at the regional scale. Based on these results and interpretations, one might argue that large scale modelling studies implementing the Q_{10} of SOC decomposition extracted from the plot scale, without considering its spatial variability and factors affecting that spatial variability, may over- or underestimate the SOC decomposition response in a warming world.

Seasonal variations were measured in the temperature sensitivities of enzymes catalyzing labile and complex organic C, β-glucosidase and phenoloxidase, at the plot scale in two geographically different areas (Table 5.1, S5.2, Chapter 5). Similar to our results, other studies have also found a strong seasonal dependency of the temperature sensitive response of hydrolytic and oxidative enzymes belonging to C, N and P cycling (Jing et al., 2014; Machmuller et al., 2016). These results could be explained by strong seasonal substrate (plant root and root exudates) and nutrient variations, and by different temperature responses of isoenzymes (Bárta et al., 2014; Jing et al., 2014; Machmuller et al., 2016; Waldrop et al., 2017). Furthermore, these results could also be associated with alterations in soil microbial community composition as a response to temperature variation. In the microcosm experiment (Chapter 6), an increase in temperature influenced abundances of soil fungi differently than bacteria when supplied with labile C substrate; fungal and gram-positive bacterial abundances decreased with temperature, while abundance of gram-negative bacteria increased (Figs. 6.2 and 6.3). Interpreting this observed microbial community response to temperature and substrate variations over the season, altered microbial community composition as a response to seasonal temperature variation might have affected enzyme / isoenzyme pool

dynamics, that in turn, have different temperature sensitivities. Indeed, studies have associated microbial community composition with production of enzymes that vary in their physical flexibility to temperature variation, hence different sensitivities (Alster et al., 2016). Therefore, in the context of climate change, microbial community composition may indirectly control SOC decomposition dynamics by shaping soil enzyme patterns.

Increasing temperature is often coupled with reduced soil moisture content and, although these two variables are among the most important factors affecting microbial SOC decomposition in a warming climate, many studies have focused only on the temperature effect (Yuste et al., 2007). In the framework of this thesis, the response of β-glucosidase potential activity to moisture variability was investigated by fitting an asymptotic model to measured activities at four moisture levels (Chapter 5). β-glucosidase potential activity exhibited a positive moisture sensitivity response; activity increased with increasing soil water content (Fig. 5.2), and this result is similar to what was observed by Steinweg *et al.,* (2012). Eliminating the water limitation may have increased enzyme diffusion to substrate and *vice versa* (Davidson and Janssens, 2006). Critically, however, the moisture sensitivity of microbial C dynamics in a dry soil environment, a condition that could potentially hamper the observed temperature sensitivity response of extracellular enzymes, was not investigated in the present study. For example, enzyme adsorption to clay particles in dry soils may affect their conformation, hence their catalytic efficiency (Kandeler, 1990). On the other hand, such enzyme stabilization mechanisms may also increase enzyme turnover time; dry soils could then harbor a larger enzyme pool (Steinweg et al., 2012), leading to potentially different responses to environmental perturbations such as temperature.

Decades of research on extracellular enzyme activity informed our understanding of the crucial links between microbial community composition (e.g., bacteria and fungi) and soil functions through the use of extracellular enzymes as proxies for those linkages (Kandeler, 1990; Sinsabaugh et al., 2008; German et al., 2011; Waldrop et al., 2017). Studies have also previously investigated *in situ* enzyme potentials and their respective controls (Wallenstein et al., 2009; Steinweg et al., 2012; Bárta et al., 2014). However, a relationship between *in situ* enzyme potentials and measured microbial response factors such as soil respiration, was not established, even though more than half of soil respiration comes from enzyme-mediated SOC decomposition (Chen et al., 2017). Therefore, this missing-link was investigated in the present thesis (Chapter 5) in which the aim was

to test whether or not soil temperature and moisture-regulated extracellular enzyme potentials explain the seasonal variations in soil respiration reported in different studies (Reichstein et al., 2003). Modelled *in situ* enzyme potentials successfully explained seasonal variation in soil respiration, with model efficiencies of up to 78 % and 76 % for β-glucosidase and xylanase, respectively (Table 5.4). The observed interaction between *in situ* enzyme potential and soil respiration flux was non-linear, which may be attributed to high microbial / substrate spatial variations, microbial thermal acclimation, and physiological adjustments (Resat et al., 2012; Frey et al., 2013). The superiority of an enzyme-based model in explaining soil respiration efflux as compared to traditional soil temperature–soil respiration relationships was also tested; enzyme-based models performed better than simple soil respiration models as a function of *in situ* soil temperature (Table 5.4). Allison *et al.,* (2010) demonstrated that adding microbial mechanisms (enzyme temperature sensitivity) could explain soil respiration responses to warming better than the conventional model with no explicit consideration of microbial mechanisms. Therefore, the inclusion of extracellular enzyme sensitivity to environmental disturbances (e.g., temperature and moisture alterations) in C modelling dynamics may have the potential to improve soil carbon-climate feedbacks.

Seasonal variations in enzyme potentials could be explained by regulation of extracellular enzymes through temperature and substrate alterations (Fig. 5.1, Table 5.1; see discussion above). Also higher *in situ* enzyme potential of soils under vegetation influence as compared to bare fallow soils, despite higher soil temperatures in the bare fallow soils (Fig. 5.4 c,d), supports the claim that temperature may not be the sole regulator of microbial C utilization dynamics; substrate quantity and quality are also important. It is, however, still unclear the extent to which soil temperature influences microbial community composition and abundance, and the extent to which bacteria and fungi differ in their respiration responses to variations in temperature and substrate quality (Malcolm et al., 2008). The hypothesis that the interactive effects of temperature and substrate quality will strongly influence the abundance and inter-/intra-specific competition between microbial key players was tested in a microcosm experiment (Chapter 6). Increasing temperature influenced soil microbial biomass negatively with lowest fungal and bacterial biomass recorded at highest temperatures, and the effect was similar in substrates of varying complexity (Fig. 6.2 a,b; Fig. 6.3). Such a decrease in microbial biomass with soil warming has been observed in other studies, perhaps due to increased metabolic stress at high temperatures (Zogg et al., 1997; Waldrop

and Firestone, 2004). The observed decline in microbial biomass may also be explained by altered microbial growth efficiencies (Schimel, 2013). Temperature increase may have stimulated microbial activity, as observed through the measured increase in cumulative respiration (Fig. 6.1), but affected their growth efficiencies strongly negatively, leading to a net decline in their biomass. Therefore, in the context of climate change, decomposition of SOC might not be accelerated by soil warming due to reduced microbial population size (Allison et al., 2010). However, under prolonged warming, microbial thermal adaptation might provide a different outcome; Frey *et al.,* (2013) observed that chronic warming led to adjustments in microbial substrate use efficiency to a higher level, especially for recalcitrant substrates. In this context, soil warming might provide positive feedback to climate change. Therefore, which pathways of SOC decomposition dynamics dominate under soil warming would strongly depend on either adaptation of existing microbial communities or shifts in microbial community composition towards more efficient individuals / groups (Frey et al., 2013; Schimel, 2013).

Efficient microbial utilization of C substrate against environmental stress, such as temperature change, determines the rate of microbial C turnover and C sequestration, although C quality may influence microbial community composition (Goldfarb et al., 2011; Sinsabaugh et al., 2017). In the present thesis, temperature influenced microbial communities' inter- and intra-specific competition when supplied with C substrates of varying quality (Chapter 6). Fungi and gram-positive bacteria generally exhibited similar responses to temperature variations; abundance of fungi and gram-positive bacteria decreased with increasing temperature and addition of labile and recalcitrant substrate increased their abundances compared to un-amended controls (Fig. 6.2 c,d; Fig. 6.3 a,b). However, gram-negative bacterial abundance increased continuously with increasing temperature in soils amended with labile C substrate (Fig. 6.2 e,f). These results hint to adaptation responses of microbial communities to induced environmental stress. Soil fungi and gram-positive bacteria generally exhibit oligotrophic life strategies and are associated with decomposition of complex substrates (Fanin et al., 2014; Koranda et al., 2014; Geyer et al., 2016); however, to cope with induced temperature stress, they have broadened their substrate utilization spectrum by consuming both labile and recalcitrant substrates. Copiotrophic gram-negative bacteria, although they are known to have low substrate utilization efficiency and low ability to cope with stress conditions, exhibited rapid substrate utilization rates under C rich conditions (Fierer et al., 2007; Geyer et al., 2016). This may have provided them with a competitive advantage over gram-positive

bacteria/fungi, leading to higher abundances. However, increased abundance of gram-negative bacteria under future soil warming, couple with their higher utilization of labile C, might be of less concern since most of the labile C present in soil may be associated with soil organic matter and minerals, rendering its decomposition difficult (Colman and Schimel, 2013). The broad substrate utilization spectrum of oligotrophs, combined with their high substrate use efficiencies, low biodegradability of cell walls, and comparatively slow turnover times, could be important for SOC sequestration in a warming world (Six et al., 2006; Rousk and Bååth, 2007).

8.1.1 Microbial community responses in the context of site-specificity and SOC quality

Microbial community adaptation to climatic and edaphic properties may produce site-specific responses to environmental alterations. For example, cold-adapted microbial communities may respire more C at high temperatures due to greater temperature sensitivity than warm-adapted microbial communities, and this effect could arise through changes in microbial enzyme expressions (Bradford, 2013; Schindlbacher et al., 2011). In the present thesis, two study areas and land-uses (bare fallow and soils influenced by vegetation) were investigated under the hypothesis that differences in climatic and edaphic properties of two study areas will modify the temperature sensitivity of microbial communities and their functions. Bare fallow soils were investigated under the assumption that these soils would have been depleted in labile organic C and therefore be inhabited by distinct microbial communities adapted to reduced substrate quality. Soils under the influence of vegetation had higher microbial biomass (C_{mic} and total PLFA content) than soils left fallow for four years and this effect also persisted over seasons (Fig. S5.2; Fig. 6.2 a,b, Fig. S6.3 a,b). Also, C rich soils of the Swabian Alb had higher microbial biomass than the Kraichgau, irrespective of the spatial scale (Chapters 5, 6 and 7). Site-specific and land-use specific differences were also observed in the activities of extracellular enzymes; potential activities of β-glucosidase and xylanase were higher in soils influenced by vegetation than bare fallow soils and also higher in the Swabian Alb than the Kraichgau (Chapter 5, Fig. 5.1 and Chapter 7; Fig. 7.2). However, these differences were only in absolute terms; patterns of observed responses were similar in both areas and land-uses and may have been associated with soil carbon content, one of the most important factors regulating soil microbial growth. At low carbon concentrations, in the absence of plant-related C inputs to bare fallow soils, extracellular enzyme production by microbial decomposers is also downregulated (German et al., 2011; Demoling et al., 2007). Furthermore,

contrary to our hypothesis, regional and land-use specificity was absent, except for absolute differences, in microbial communities' responses to temperature variations when supplied with substrates of varying complexity (e.g., Figs. 6.2, S6.3, S6.9) and no clear differences were observed in the response of investigated bacterial taxa (Figs. 6.5, S6.6). It follows that region and / or land-use might have smaller impacts on microbial abundance and community composition than temperature and substrate quality. These results could be associated either with substrate use plasticity of soil microorganisms, which has been observed to change depending upon environmental conditions (Morrissey et al., 2017), or with functional redundancy of soil microorganisms. Microbial communities adapted to certain regions / land-uses may contain taxa that are functionally redundant with taxa in other regions / land-uses or taxa in adapted communities might function differently but result the in same process rates when observed from the community level (Allison and Martiny, 2008). Nevertheless, microbial physiological differences and their ability to behave differently under different environmental perturbations may determine the fate of SOC decomposition in a warming world, with region and land-use playing a secondary role. Therefore, associating temperature and substrate quality sensitive responses of microbial key players based on their physiology and life strategies, i.e., copiotrophs *vs.* oligotrophs, may well improve C models at large scales.

8.2 Scale-specificity of factors controlling microbial SOC dynamics

The dynamics of microbial SOC processing at large scales are poorly understood, a result of which is the large spread in global SOC stock predictions under a changing climate (Bond-Lamberty and Thomson, 2010; Todd-Brown et al., 2013). However, it is still not completely clear which factors controlling SOC decomposition at the plot scale are also important at larger scales. For example, at the fine scale of mm to m, microbial community composition may be important due to its functional specificity, whereas at the coarse scale, abiotic factors, including temperature and moisture, may be more important (Classen et al., 2015). Therefore, one aim of the present thesis was to identify factors controlling microbial SOC decomposition dynamics at two large spatial scales; the landscape scale, representing two individual areas (the Kraichgau and the Swabian Alb), and the regional scale, representing combined data from both areas (Chapter 7). The hypothesis was that from a set of biotic, physical, and chemical soil properties, the quality of SOC would be

the primary control on microbial decomposition processes (basal respiration and its temperature sensitivity) due to its lability and relevance for microbially mediated decomposition.

Different factors explained microbial SOC decomposition and its temperature sensitivity at different spatial scales (Chapter 7). Soil microbial biomass (C_{mic}) explained soil basal respiration independent of spatial scale, while soil C:N ratio and SOC quantity were scale-dependent factors (Table 7.2). Association of C_{mic} and C:N (proxy for SOC quality) as explanatory factors for soil respiration at large scales has also been shown by other studies (Colman and Schimel, 2013; Xu et al., 2016). From our results, microbial biomass emerged as an integrative proxy for SOC decomposition dynamics; i.e., it integrated the effects of all other variables (Bailey et al., 2018). Liu *et al.* (2018) identified a unique role for soil microbial biomass and microbial community composition in predicting soil respiration rates. Based on our results, it may be that that microbial biomass pool size and SOC quality limit SOC decomposition when landscapes with different SOC content and climatic conditions are investigated in a single model. However, in landscapes of low SOC content such as the Kraichgau, it is the quantity of SOC that limits its decomposition by microbial decomposers. Furthermore, temperature sensitivity of SOC decomposition was successfully explained by soil texture and this control was independent of spatial scale, whereas C_{mic} explained the Q_{10} of SOC decomposition at the regional scale (Table 7.2). Variables explaining temperature sensitivities of extracellular enzymes were scale-specific (Table 7.2). In a study by Trivedi *et al.* (2016), microbial functional genes provided a strong explanation for observed soil functions even though many other biotic and abiotic processes were considered. Therefore, adding scale-specific variables and variables that are independent of the spatial scale as controls on process rates in regional scale C dynamics models, providing further input to global scale models, may improve our understanding of the fate of SOC in a warming world.

8.3 Conclusions and perspectives

This thesis provides new insights into the critical role soil microorganisms play in SOC decomposition dynamics and demonstrates the extent to which C turnover may be affected by soil microbial communities as a response to climate change at large scales. It was shown that temperature and quality of soil organic C play central roles in controlling microbial SOC decomposition dynamics irrespective of land-use and spatial scale. From the plot to the regional scale and from point measurement to measurements spanning seasons, this thesis provided evidence that the temperature sensitivity of SOC varies at both spatial and temporal scales and decreases with increasing soil temperature. This contradicts the assumption used in most CN models that it is constant (Q_{10} = 2). Therefore, the observed variance in SOC Q_{10} in this study should be added to CN models to potentially improve their predictive power. Furthermore, analysis of extracellular enzymes, as proxies for varying lability in SOC pools, indicated that the labile SOC pool exhibited lower temperature sensitivities than the recalcitrant pool. This could have important implications for predictions of the relative SOC pool size estimations in the context of climate change. Although soil warming reduced the abundances of soil bacteria and fungi, it stimulated their respiration responses. Together these results indicate that microbial SOC decomposition may provide a positive feedback to climate change, at least at the scale investigated here. Soil fungi are usually associated with decomposition of complex SOC while bacteria prefer labile SOC; however, similar utilization of labile and recalcitrant C by fungi was observed. Additionally, soil warming selected for dominance of gram-negative bacteria over gram-positive bacteria. Therefore, with shifts in microbial community functional traits, the fungal/bacterial ratio may change in a warming world, with important implications for SOC sequestration.

The large spread in predictions of SOC stock sizes observed at the global scale (Hararuk et al., 2015) could perhaps be improved by adding microbial controls on SOC decomposition in C models at the regional scale. By focusing on the soil microbial perspective, this thesis provides an improved understanding of the physicochemical and biological controls of SOC decomposition dynamics at large spatial scales. To improve regional scale climate change projections, efforts are being made that provide input for global scale model improvements. Models are run, for example, in the framework of EURO–CORDEX (coordinated downscaling experiment – European domain), at a spatial resolution of about 12 km (http://www.euro-cordex.net/060374/index.php.en). This makes

the results of this thesis highly applicable and timely, as the presently investigated landscapes are approx. 95 km apart from each other. Explanatory factors of microbially medicated SOC decomposition common at the landscape and/or at regional scale extracted in this study may be directly used for SOC model optimization.

Furthermore, this thesis highlights the potential for extracellular enzyme potentials and their temperature sensitivities to be used as proxies for different SOC pools, which may improve the predictive power of C dynamics models. The validity of *in situ* enzyme potentials *vs.* soil respiration relationships for N and P cycling in similar and in different ecosystems should be tested in future studies. There are, however, some methodological issues related to this. One problem with measuring *in situ* enzyme potentials is that lab-based assays also measure the activity of stabilized enzymes, through disruption of aggregates, that usually form complexes with organic matter or clays and these enzymes may not be active under *in situ* conditions (Wallenstein and Weintraub, 2008). With our modelled *in situ* enzyme potentials, even though we considered control of *in situ* temperature and moisture, the above stated problem may still exist. Therefore, there is a need to combine lab-based enzyme assays (temperature and moisture sensitivity measurements) with *in situ* measurement methods, for instance enzyme zymography, which may provide a more accurate assessment of environmental alterations to enzyme potentials at large scales.

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Curriculum Vitae

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Education

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2009 – 2011	M.Sc. in Environmental Protection and Agricultural Food Production University of Hohenheim, Stuttgart, Germany
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Work Experience

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Presentations and publications

I supervised a bachelor thesis in the framework of my PhD. Parts of the PhD thesis and other projects were published or presented at national and international conferences given below:

Oral presentations

Kandeler, E., Spohn, M., Reinhhardt, D., Boeddinghaus, R., Gebala, A., Ali, R.S., Marhan, S., Poll, C. (2016) From visualization of enzyme activities to *in-situ* process rates in soils. Enzymes in the Environment (Bangor, United Kingdom).

Demyan, M.S., Rasche, F., Marohn, C., Mirzaeitalarposhti, R., Funkuin, Y.N., Ali, R.S., Priesack, E., Högy, P., Ingwersen, J., Schulz, E., Kätterer, T., Wizemann, H.-D., Müller, T., Cadisch, G. (2015) Spectroscopic and thermo-spectroscopic approaches for pool parameterization of SOM models. 5th Internation Symposium on Soil Organic Matter (Göttingen, Germany).

Poll, C., Ali, R.S., Demyan, S., Funkuin, Y.N., Kandeler, E. (2014) Modelling *in situ* enzyme potential of soils: a tool to predict soil respiration from agricultural soils. The Ecological Society of Germany, Austria and Switzerland; method workshop (Halle, Germany).

Demyan, S., Marohn, C., Rasche, F., Mirzaeitalarposhti, R., Funkuin, Y.N., Ali, R.S., Högy, P., Ingwersen, J., Wizemann, H.-D., Müller, T., Cadisch, G. (2014) Implications of spectroscopic and thermo-spectroscopic approaches for pool parameterization of SOM models. EGU General Assembly (Vienna, Austria).

Ali, R.S., Poll, C., Kandeler, E. (2013) Microbial regulation of organic matter decomposition at the regional scale – temperature sensitivity of soil enzymes. German Soil Science Society conference (Rostock, Germany).

Ali, R.S., Poll, C., Kandeler, E. (2013) Microbial regulation of organic matter decomposition at the regional scale. International course in Microbial Ecology (Venice, Italy).

Poll, C., Ali, R.S., Ditterich, F., Pagel, H., Ingwersen, J., Marhan, S., Streck, T., Kandeler, E. (2013) Functions of microbial communities in soils: Impact of anthropization and remediation-soil enzymes as a tool to measure microbial functioning. Summer school, (Nancy, France).

Poster presentations

Ali, R.S., Poll, C., Kandeler, E. (2017) Microbial growth response to substrate complexity under different temperature regimes. Conference of the German Soil Science Society, (Göttingen, Germany).

Ali, R.S., Kandeler, E., Marhan, S., Demyan, M.S., Ingwersen, J., Mirzaeitalarposhti, R., Rasche, F., Cadisch, G., Poll, C. (2017) Controls on microbially regulated soil organic carbon decomposition at the regional scale. 6th International Symposium on Soil Organic Matter (Harpenden, UK).

Ali, R.S., Kandeler, E., Poll, C., Ingwersen, J., Demyan, M.S., Funkuin, Y.N., Wizemann, H-D. (2016) Modelling *in situ* activities of enzymes as a tool to explain seasonal variation of soil respiration from agro-ecosystems. Enzymes in the Environment; Ecology, Activity and Applications (Bangor, UK).

Poll, C., Ali, R.S. (2016) Moisture and temperature sensitivity of extracellular enzymes. Enzymes in the Environment (Bangor, Wales, United Kingdom).

Ali, R.S., Poll, C., Kandeler, E. (2015) Effects of substrate complexity and temperature on growth of different microbial groups. Ecology of Soil Microorganisms (Prague, Czech Republic).

Ali, R.S., Poll, C., Ingwersen, J., Deyman, M.S., Kandeler, E. (2015) Temperature sensitivity of microbial-mediated soil organic matter decomposition: Predicting factors at the regional scale. Conference of the German Soil Science Society (Munich, Germany).

Ali, R.S., Poll, C., Demyan, S., Funkuin, Y.N., Ingwersen, J., Wizemann, H-D., Kandeler, E. (2014) Modelling *in situ* enzyme potential of soils: a tool to predict soil respiration from agricultural fields. EGU General Assembly (Vienna, Austria).

Ali, R.S., Poll, C., Demyan, S., Funkuin, Y.N., Ingwersen, J., Wizemann, H.-D., Kandeler, E. (2014) Modelling *in situ* enzyme potential of soils: a tool to predict soil respiration from agricultural fields. Global Soil Biodiversity Conference (Dijon, France).

Peer-reviewed publications

Ali, R.S., Poll, C., Kandeler, E. (2018) Dynamics of soil respiration and microbial communities: Interactive controls of temperature and substrate quality. Soil Biology and Biochemistry 127, 60 – 70.

Ali, R.S.**,** Kandeler, E., Marhan, S., Demyan, M.S., Ingwersen J., Mirzaeitalarposhti, R., Rasche, F., Cadisch, G., Poll, C. (2018) Controls on microbially regulated soil organic carbon decomposition at the regional scale. Soil Biology and Biochemistry 118, 59 – 68.

Demyan, M.S., Ingwersen, J., Funkuin, Y.N., Ali, R.S., Mirzaeitalarposhti, R., Rasche, F., Poll, C., Müller, T., Streck, T., Kandeler, E. (2016) Partitioning of ecosystem respiration in winter wheat and silage maize - modeling seasonal temperature effects. Agriculture, Ecosystem and Environment 224, 131 – 144.

Ali, R. S., Ingwersen, J., Demyan, M. S., Funkuin, Y. N., Wizemann, H.-D., Kandeler, E., Poll, C. (2015) Modelling *in situ* activities of enzymes as a tool to explain seasonal variation of soil respiration from agro-ecosystems. Soil Biology and Biochemistry 81, 291 – 303.

Supervised bachelor thesis

"Methodenentwicklung (MUF) und die Auswirkungen der Feuchtigkeit auf die Bodenenzymaktivität – β-glucosidase" by Oxana Schuldeis (2013).

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Appendix – I

Supplementary data – Chapter 5

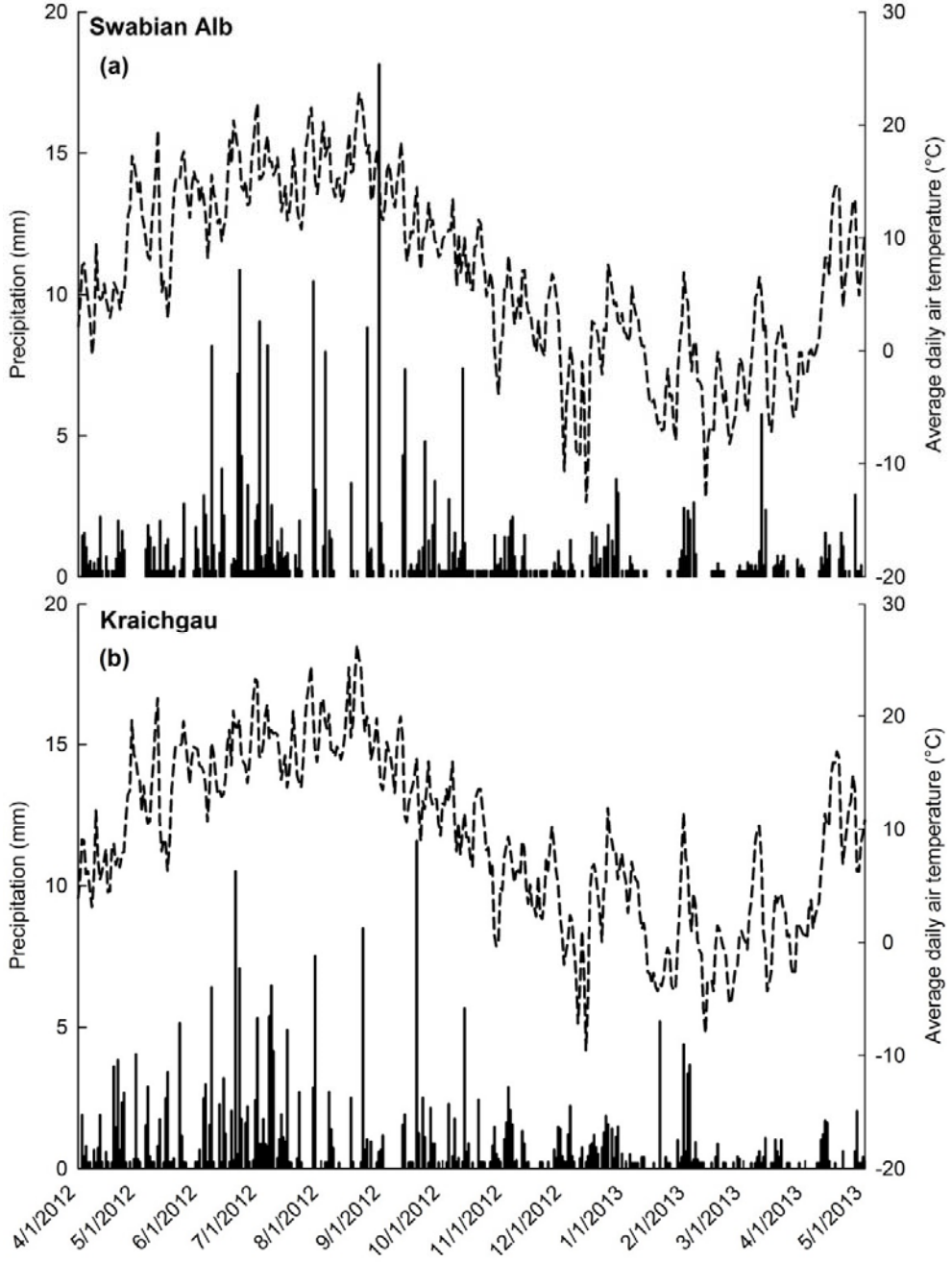

Fig. S5.1 Average daily air temperature and precipitation data recorded at the Swabian Alb (a) and the Kraichgau (b) study sites. The data were recorded in half hourly resolution.

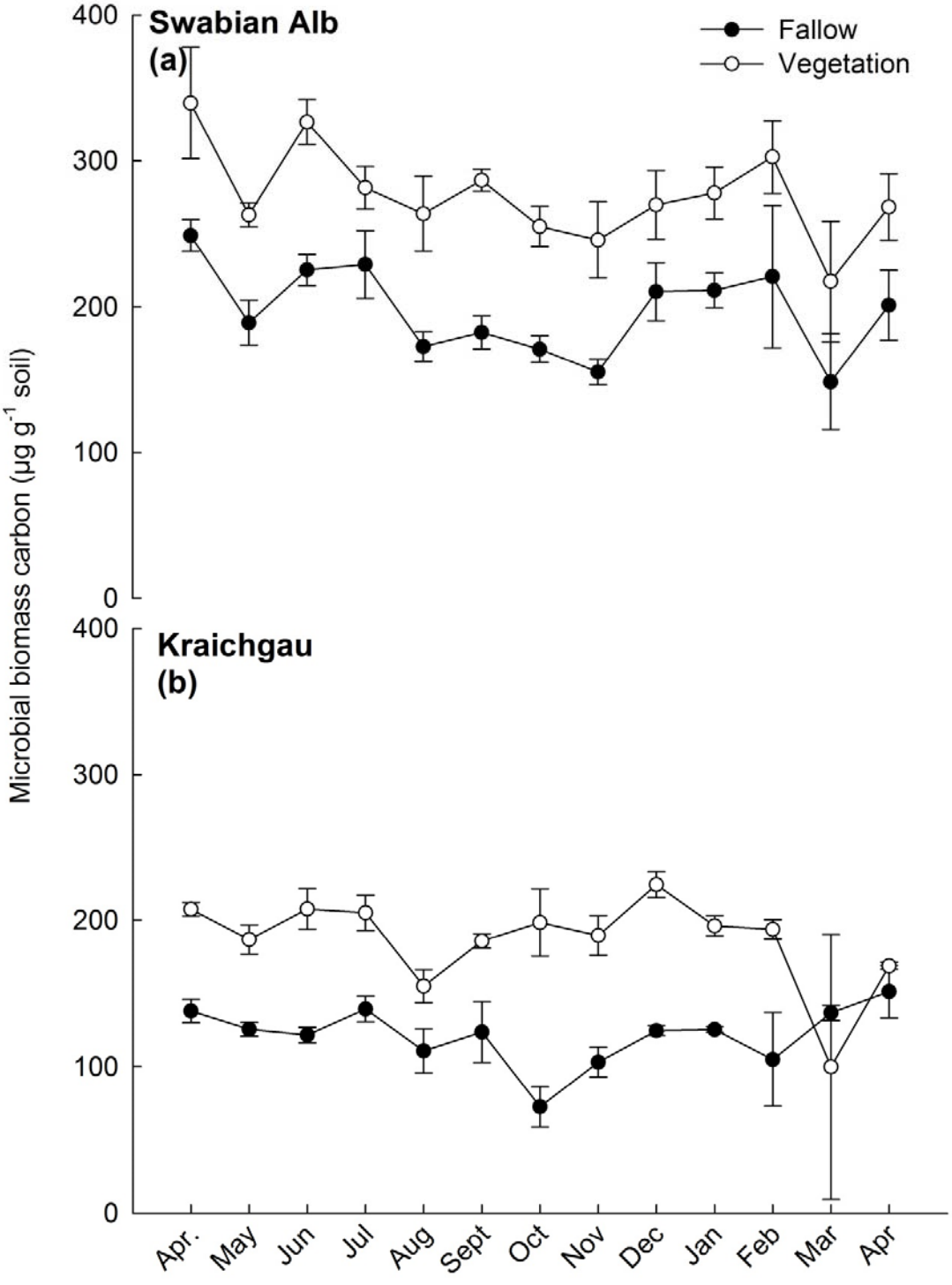

Fig. S5.2 Average microbial biomass measured from the fallow and vegetation plots of both study sites. Bars represent the standard error (n = 3)

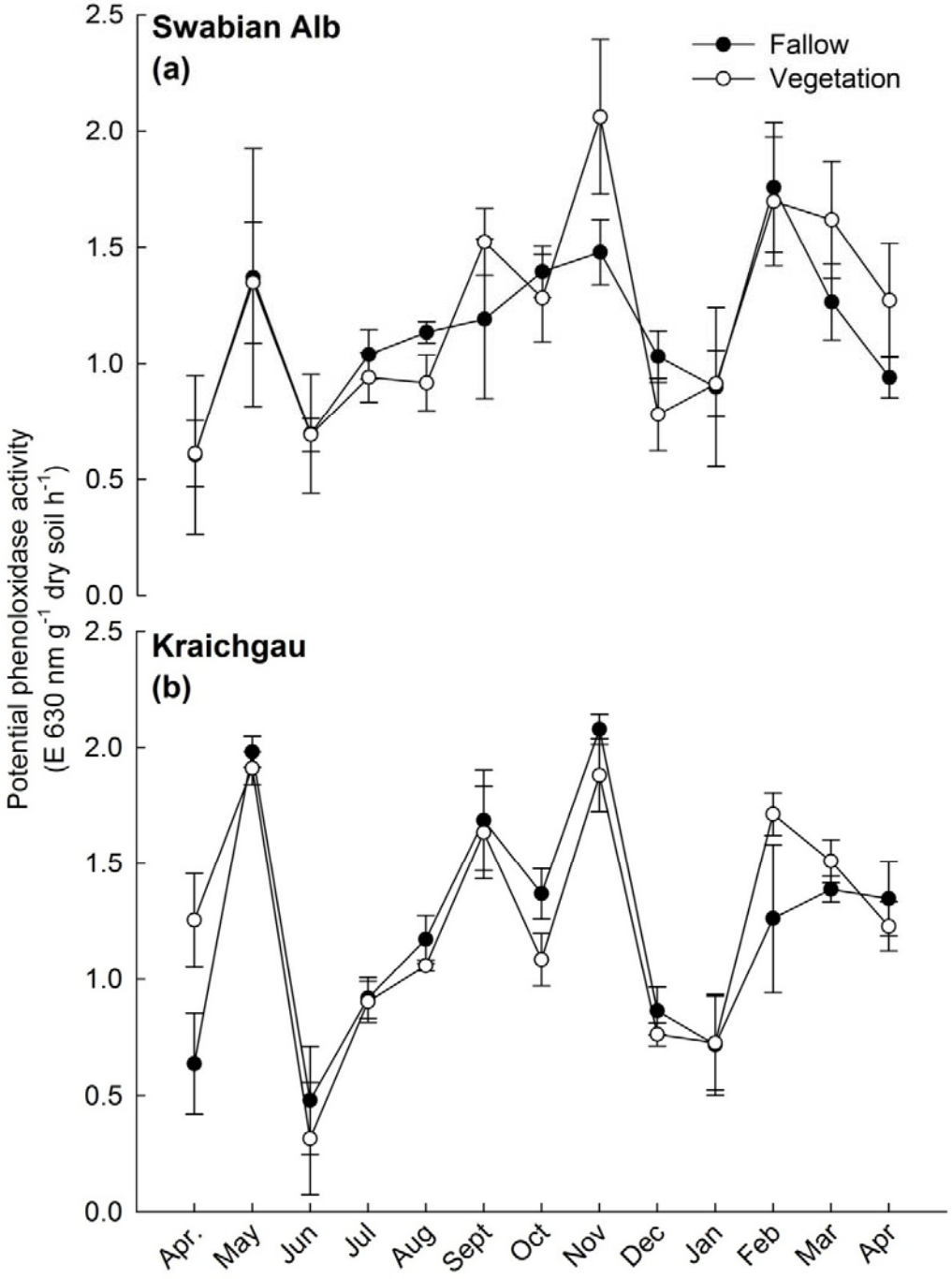

Fig. S5.3 Potential Phenoloxidase activities in the Swabian Alb (a) and the Kraichgau region (b) measured at different sampling dates (from April 2012 till April 2013). Bars represent standard error (n = 3).

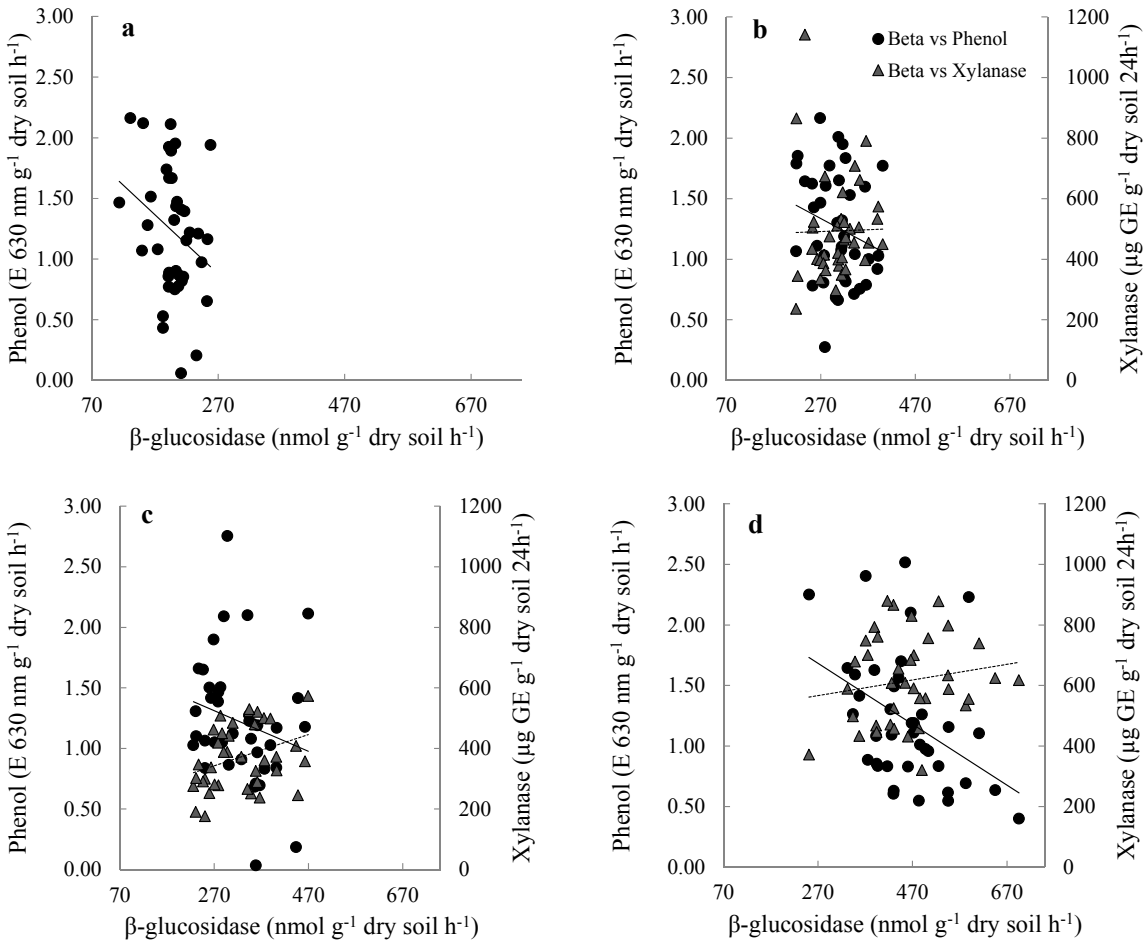

Fig. S5.4 Potential β-glucosidase activities in relation to xylanase and phenoloxidase (Phenol) activities. "a" represents activity relation from Kraichgau fallow plots, "b" Kraichgau vegetation plots, "c" Swabian Alb fallow plots and "d" represents enzyme activity relation from Swabian Alb vegetation plots, respectively (n = 39).

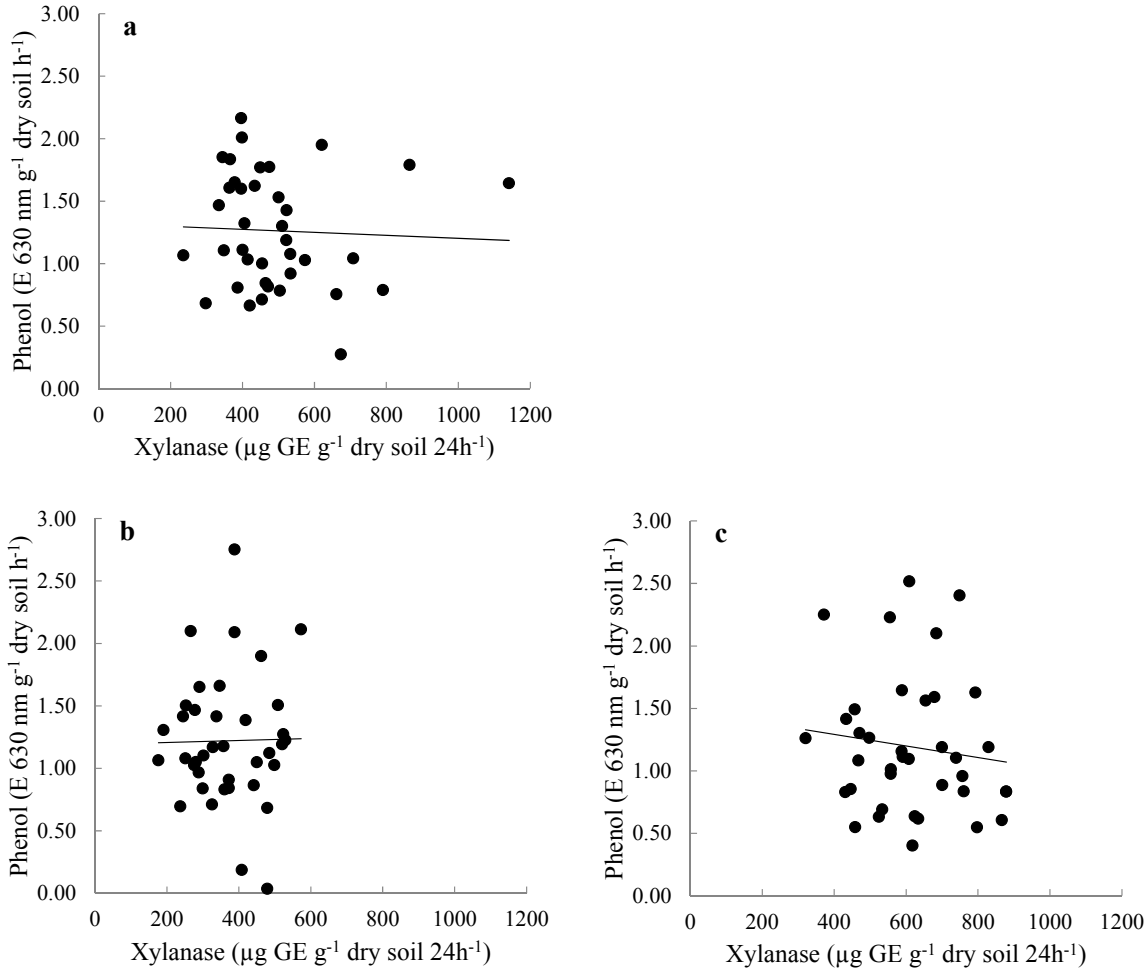

Fig. S5.5 Potential xylanase activities in relation to phenoloxidase (Phenol) activities. "a" represents activity relation from Kraichgau vegetation plots, "b" Swabian Alb fallow plots and "c" represents enzyme activity relation from Swabian Alb vegetation plots, respectively (n = 39).

Table S5.1 Crop type as well as vegetation period of the selected vegetation plots, (covering the sampling dates starting April 2012 to April 2013) in both study regions. In each region, three vegetated plots were selected.

Region	Plot	Crop	Vegetation period
Swabian Alb	Vegetation 1	Spring Barley *(Hordeum vulgare*)	25.03.2012 - 15.08.2012
	Vegetation 2	Silage Maize *(Zea mays*)	28.04.2012 - 07.10.2012
	Vegetation 3	Winter Barley *(Hordeum vulgare*)	22.09.2011 - 26.07.2012
	Vegetation 1	Winter Rape *(Brassica napus)*	28.08.2012 - 22.08.2013
	Vegetation 2	Winter Barley *(Hordeum vulgare*)	15.10.2012 - 02.08.2013
	Vegetation 3	Cover Crop *	01.08.2012 - 12.01.2013
Kraichgau	Vegetation 1	Winter Rape *(Brassica napus)*	22.08.2011 - 20.07.2012
	Vegetation 2	Energy Maize *(Zea mays)*	01.05.2012 - 18.09.2012
	Vegetation 3	Winter Wheat *(Triticum aestivum)*	17.10.2011 - 01.08.2012
	Vegetation 1	Winter Wheat *(Triticum aestivum)*	17.10.2012 - 04.08.2013
	Vegetation 2	Winter Wheat *(Triticum aestivum)*	26.10.2012 - 05.08.2013
	Vegetation 3	Winter Rape *(Brassica napus)*	24.08.2012 - 02.08.2013

*Common vetch *(Vicia sativa)* 60%, Berseem clover *(Trifolium alexandrinum)* 25% Phacelia 7.5%, Sunflower *(Helianthus annuus)*7.5%

Table S5.2 Q_{10} values of phenoloxidase measured at different laboratory incubation temperatures (starting from April 2012 till April 2013). Values represent average of field replicates (n = 3) and standard error is given in parenthesis

Month	Swabian Alb		Kraichgau	
	Fallow	Vegetation	Fallow	Vegetation
Apr	0.82 (0.14)	0.14 (0.09)	1.05 (0.09)	0.84 (0.04)
May	1.56 (0.19)	0.19 (0.30)	1.38 (0.10)	1.28 (0.26)
Jun	1.55 (0.25)	0.25 (0.06)	0.55 (0.15)	0.88 (0.20)
Jul	1.23 (0.06)	0.06 (0.22)	0.95 (0.06)	0.90 (0.02)
Aug	1.22 (0.15)	0.15 (0.10)	1.05 (0.09)	0.84 (0.04)
Sept	1.05 (0.19)	0.19 (0.15)	1.32 (0.08)	1.24 (0.11)
Oct	1.43 (0.08)	0.08 (0.04)	1.29 (0.02)	1.17 (0.03)
Nov	1.19 (0.12)	0.12 (0.13)	1.14 (0.13)	1.71 (0.58)
Dec	1.26 (0.14)	0.14 (0.12)	0.96 (0.05)	0.99 (0.20)
Jan	1.25 (0.09)	0.09 (0.12)	1.00 (0.11)	0.86 (0.01)
Feb	1.87 (0.22)	0.22 (0.52)	1.10 (0.08)	1.40 (0.05)
Mar	1.52 (0.28)	0.28 (1.06)	1.21 (0.15)	1.25 (0.13)
Apr	1.16 (0.07)	0.07 (0.08)	1.12 (0.05)	1.09 (0.08)

Table S5.3 Q_{10} of individual plots (fallow and vegetation) from both study regions together with coefficient of exponential model applied (*a*), calculated activity at 6°C (R_6), k_0 (β-glucosidase - nmol g^{-1} h^{-1} and xylanase–µg GE g^{-1} $24h^{-1}$) as well as model efficiencies.

β-GLUCOSIDASE	**Kraichgau**						**Swabian Alb**				
	Fallow Plot						**Fallow Plot**				
Month	*Plot*	R_6	k_0	*a*	Q_{10}	*EF*	R_6	k_0	*a*	Q_{10}	*EF*
Apr, 2012	fallow1	44.66	29.47	0.0693	2.0	0.86	84.91	57.87	0.0639	1.9	0.86
	fallow2	47.40	33.17	0.0595	1.8	0.93	106.37	73.37	0.0619	1.9	0.82
	fallow3	69.99	51.29	0.0518	1.7	0.89	93.23	64.35	0.0618	1.9	0.84
May, 2012	fallow1	54.67	39.21	0.0554	1.7	0.86	91.59	69.75	0.0454	1.6	0.86
	fallow2	63.87	45.15	0.0578	1.8	0.93	79.78	59.28	0.0495	1.6	0.82
	fallow3	64.48	48.90	0.0461	1.6	0.89	83.19	59.56	0.0557	1.7	0.84
June, 2012	fallow1	50.91	37.43	0.0513	1.7	0.86	85.89	60.03	0.0597	1.8	0.86
	fallow2	53.57	38.49	0.0551	1.7	0.93	91.60	64.10	0.0595	1.8	0.82
	fallow3	51.91	37.03	0.0563	1.8	0.89	101.97	74.55	0.0522	1.7	0.84
July, 2012	fallow1	58.46	44.23	0.0465	1.6	0.86	93.62	65.91	0.0585	1.8	0.86
	fallow2	54.95	39.24	0.0561	1.8	0.93	89.52	61.67	0.0621	1.9	0.82
	fallow3	56.89	41.34	0.0532	1.7	0.89	73.21	51.04	0.0601	1.8	0.84
Aug, 2012	fallow1	52.84	37.58	0.0568	1.8	0.86	77.89	55.10	0.0577	1.8	0.86
	fallow2	54.67	38.14	0.0600	1.8	0.93	82.11	57.15	0.0604	1.8	0.82
	fallow3	57.71	40.78	0.0579	1.8	0.89	91.76	65.14	0.0571	1.8	0.84
Sept, 2012	fallow1	36.16	26.31	0.0530	1.7	0.86	87.31	65.34	0.0483	1.6	0.86
	fallow2	61.71	45.31	0.0515	1.7	0.93	96.13	69.70	0.0536	1.7	0.82
	fallow3	56.67	41.98	0.0500	1.6	0.89	73.65	53.78	0.0524	1.7	0.84
Oct, 2012	fallow1	40.73	28.90	0.0572	1.8	0.86	81.95	59.84	0.0524	1.7	0.86
	fallow2	58.32	42.18	0.0540	1.7	0.93	122.34	86.95	0.0569	1.8	0.82
	fallow3	54.01	39.04	0.0541	1.7	0.89	63.20	44.70	0.0577	1.8	0.84
Nov, 2012	fallow1	40.84	30.05	0.0511	1.7	0.86	69.70	50.80	0.0527	1.7	0.86
	fallow2	60.54	45.36	0.0481	1.6	0.93	73.39	54.47	0.0497	1.6	0.82
	fallow3	47.24	34.87	0.0506	1.7	0.89	67.54	48.70	0.0545	1.7	0.84
Dec, 2012	fallow1	37.93	26.81	0.0578	1.8	0.86	84.82	60.14	0.0573	1.8	0.86
	fallow2	58.59	43.02	0.0515	1.7	0.93	88.38	59.45	0.0661	1.9	0.82
	fallow3	61.57	46.44	0.0470	1.6	0.89	58.92	41.93	0.0567	1.8	0.84
Jan, 2013	fallow1	52.20	37.08	0.0570	1.8	0.86	80.53	58.38	0.0536	1.7	0.86
	fallow2	56.47	41.38	0.0518	1.7	0.93	112.88	85.19	0.0469	1.6	0.82
	fallow3	55.37	40.60	0.0517	1.7	0.89	61.76	44.78	0.0536	1.7	0.84
Feb, 2013	fallow1	58.03	43.35	0.0486	1.6	0.86	93.27	62.29	0.0673	2.0	0.86
	fallow2	54.62	38.87	0.0567	1.8	0.93	109.75	77.78	0.0574	1.8	0.82

	fallow3	64.06	45.89	0.0556	1.7	0.89	84.39	59.62	0.0579	1.8	0.84
Mar, 2013	fallow1	38.06	25.63	0.0659	1.9	0.86	79.54	56.94	0.0557	1.7	0.86
	fallow2	52.76	36.31	0.0623	1.9	0.93	73.94	53.77	0.0531	1.7	0.82
	fallow3	51.33	34.26	0.0674	2.0	0.89	70.14	48.91	0.0601	1.8	0.84
Apr, 2013	fallow1	46.69	32.77	0.0590	1.8	0.86	67.43	46.34	0.0625	1.9	0.86
	fallow2	65.12	46.57	0.0559	1.7	0.93	91.62	64.46	0.0586	1.8	0.82
	fallow3	64.50	46.45	0.0547	1.7	0.89	62.11	42.90	0.0617	1.9	0.84

	Vegetation Plot						**Vegetation Plot**				
	Plot	R_6	k_0	*a*	Q_{10}	*EF*	R_6	k_0	*a*	Q_{10}	*EF*
Apr, 2012	Vegetated1	64.89	42.84	0.0692	2.0	0.91	104.47	66.21	0.0760	2.1	0.92
	Vegetated2	63.71	44.34	0.0604	1.8	0.87	166.38	115.18	0.0613	1.8	0.85
	Vegetated3	90.03	62.81	0.0600	1.8	0.89	88.73	60.95	0.0626	1.9	0.77
May, 2012	Vegetated1	72.66	51.15	0.0585	1.8	0.91	102.30	72.75	0.0568	1.8	0.92
	Vegetated2	82.58	59.69	0.0541	1.7	0.87	129.26	93.66	0.0537	1.7	0.85
	Vegetated3	94.41	69.81	0.0503	1.7	0.89	111.80	83.02	0.0496	1.6	0.77
June, 2012	Vegetated1	77.56	53.76	0.0611	1.8	0.91	115.69	77.49	0.0668	2.0	0.92
	Vegetated2	78.94	57.71	0.0522	1.7	0.87	149.20	103.16	0.0615	1.8	0.85
	Vegetated3	79.69	55.16	0.0613	1.8	0.89	115.18	82.11	0.0564	1.8	0.77
July, 2012	Vegetated1	84.83	60.70	0.0558	1.7	0.91	114.80	82.53	0.0550	1.7	0.92
	Vegetated2	95.33	71.48	0.0480	1.6	0.87	118.54	82.11	0.0612	1.8	0.85
	Vegetated3	89.14	62.34	0.0596	1.8	0.89	91.84	63.66	0.0611	1.8	0.77
Aug, 2012	Vegetated1	71.70	49.22	0.0627	1.9	0.91	107.56	71.74	0.0675	2.0	0.92
	Vegetated2	77.50	51.35	0.0686	2.0	0.87	119.05	80.46	0.0653	1.9	0.85
	Vegetated3	81.18	56.47	0.0605	1.8	0.89	143.19	98.23	0.0628	1.9	0.77
Sept, 2012	Vegetated1	73.29	51.26	0.0595	1.8	0.91	107.06	77.66	0.0535	1.7	0.92
	Vegetated2	77.83	56.59	0.0531	1.7	0.87	137.64	98.89	0.0551	1.7	0.85
	Vegetated3	74.28	55.83	0.0476	1.6	0.89	138.31	100.39	0.0534	1.7	0.77
Oct, 2012	Vegetated1	60.95	42.27	0.0610	1.8	0.91	128.25	92.59	0.0543	1.7	0.92
	Vegetated2	85.64	59.11	0.0618	1.9	0.87	120.90	85.26	0.0582	1.8	0.85
	Vegetated3	69.12	47.79	0.0615	1.8	0.89	96.52	70.27	0.0529	1.7	0.77
Nov, 2012	Vegetated1	52.48	36.31	0.0614	1.8	0.91	118.46	84.81	0.0557	1.7	0.92
	Vegetated2	75.04	54.47	0.0534	1.7	0.87	95.24	67.94	0.0563	1.8	0.85
	Vegetated3	76.44	55.79	0.0525	1.7	0.89	71.93	51.93	0.0543	1.7	0.77
Dec, 2012	Vegetated1	75.97	53.16	0.0595	1.8	0.91	125.66	87.67	0.0600	1.8	0.92
	Vegetated2	91.76	70.73	0.0434	1.5	0.87	111.67	79.85	0.0559	1.7	0.85
	Vegetated3	90.68	66.42	0.0519	1.7	0.89	101.13	70.09	0.0611	1.8	0.77
Jan, 2013	Vegetated1	86.03	61.74	0.0553	1.7	0.91	117.49	83.11	0.0577	1.8	0.92
	Vegetated2	108.16	81.29	0.0476	1.6	0.87	119.25	84.60	0.0572	1.8	0.85

	Vegetated3	70.62	51.35	0.0531	1.7	0.89	105.11	75.39	0.0554	1.7	0.77
Feb, 2013	Vegetated1	70.20	48.22	0.0626	1.9	0.91	112.43	80.44	0.0558	1.7	0.92
	Vegetated2	89.39	62.36	0.0600	1.8	0.87	144.37	101.27	0.0591	1.8	0.85
	Vegetated3	85.76	61.95	0.0542	1.7	0.89	91.76	62.31	0.0645	1.9	0.77
Mar, 2013	Vegetated1	60.15	39.71	0.0692	2.0	0.91	115.51	79.44	0.0624	1.9	0.92
	Vegetated2	76.47	50.88	0.0679	2.0	0.87	104.95	70.13	0.0672	2.0	0.85
	Vegetated3	70.41	47.44	0.0658	1.9	0.89	94.10	59.86	0.0754	2.1	0.77
Apr, 2013	Vegetated1	51.56	35.67	0.0614	1.8	0.91	114.88	80.30	0.0597	1.8	0.92
	Vegetated2	77.86	53.58	0.0623	1.9	0.87	90.13	62.36	0.0614	1.8	0.85
	Vegetated3	61.75	43.11	0.0599	1.8	0.89	69.65	46.60	0.0670	2.0	0.77

XYLANASE	**Kraichgau**						**Swabian Alb**				
	Fallow Plot						**Fallow Plot**				
	Plot	R_6	k_0	*a*	Q_{10}	*EF*	R_6	k_0	*a*	Q_{10}	*EF*
Apr, 2012	fallow1						21.56	14.07	0.0712	2.04	0.86
	fallow2						22.21	15.60	0.0589	1.80	0.84
	fallow3						22.37	14.82	0.0686	1.99	0.40
May, 2012	fallow1						20.61	13.30	0.0730	2.07	0.86
	fallow2						7.04	4.27	0.0834	2.30	0.84
	fallow3						4.00	1.96	0.1184	3.27	0.40
June, 2012	fallow1						37.44	25.42	0.0645	1.91	0.86
	fallow2						5.17	2.73	0.1065	2.90	0.84
	fallow3						21.05	13.52	0.0737	2.09	0.40
July, 2012	fallow1						67.47	50.80	0.0473	1.60	0.86
	fallow2						27.31	19.17	0.0590	1.80	0.84
	fallow3						14.07	8.82	0.0778	2.18	0.40
Aug, 2012	fallow1						128.07	109.64	0.0259	1.30	0.86
	fallow2						14.60	9.97	0.0635	1.89	0.84
	fallow3						24.72	14.86	0.0849	2.34	0.40
Sept, 2012	fallow1						8.00	4.53	0.0947	2.58	0.86
	fallow2						15.56	10.40	0.0671	1.96	0.84
	fallow3						6.21	3.48	0.0967	2.63	0.40
Oct, 2012	fallow1						11.52	6.94	0.0846	2.33	0.86
	fallow2						3.34	1.82	0.1009	2.74	0.84
	fallow3						16.74	11.46	0.0632	1.88	0.40
Nov, 2012	fallow1						90.98	74.59	0.0331	1.39	0.86
	fallow2						16.08	11.40	0.0574	1.78	0.84
	fallow3						3.44	1.85	0.1034	2.81	0.40
Dec, 2012	fallow1						38.40	27.53	0.0555	1.74	0.86
	fallow2						14.53	9.81	0.0654	1.92	0.84

	fallow3						3.81	2.18	0.0934	2.54	0.40
Jan, 2013	fallow1						15.55	9.94	0.0746	2.11	0.86
	fallow2						8.82	5.43	0.0808	2.24	0.84
	fallow3						29.26	21.30	0.0529	1.70	0.40
Feb, 2013	fallow1						79.08	60.44	0.0448	1.57	0.86
	fallow2						19.59	13.76	0.0588	1.80	0.84
	fallow3						32.55	21.30	0.0707	2.03	0.40
Mar, 2013	fallow1						9.99	5.98	0.0855	2.35	0.86
	fallow2								*Outlier*		
	fallow3						23.54	16.68	0.0574	1.77	0.40
Apr, 2013	fallow1						6.45	3.35	0.1093	2.98	0.86
	fallow2						7.61	4.34	0.0935	2.55	0.84
	fallow3						13.21	8.26	0.0782	2.19	0.40
	Kraichgau						**Swabian Alb**				
		Vegetation Plot					**Vegetation Plot**				
Apr, 2012	Vegetation1	7.924	4.53	0.0933	2.54	0.87	50.51	33.67	0.0676	1.97	0.75
	Vegetation2	13.66	8.29	0.0833	2.30	0.60	45.70	31.56	0.0617	1.85	0.85
	Vegetation3	10.22	5.87	0.0925	2.52	0.81	52.02	35.77	0.0623	1.87	0.79
May, 2012	Vegetation1	14.44	8.87	0.0811	2.25	0.87	74.50	53.17	0.0562	1.75	0.75
	Vegetation2	29.52	19.82	0.0664	1.94	0.60	19.19	12.18	0.0757	2.13	0.85
	Vegetation3	31.6	20.79	0.0698	2.01	0.81	22.16	13.05	0.0882	2.41	0.79
June, 2012	Vegetation1	26.91	17.85	0.0684	1.98	0.87	75.43	52.66	0.0599	1.82	0.75
	Vegetation2	14.03	7.90	0.0957	2.60	0.60	39.35	26.61	0.0652	1.92	0.85
	Vegetation3	42.69	28.43	0.0678	1.97	0.81	56.64	37.13	0.0704	2.02	0.79
July, 2012	Vegetation1	26.04	17.17	0.0694	2.00	0.87	118.00	85.91	0.0529	1.70	0.75
	Vegetation2	59.93	42.57	0.0570	1.77	0.60	22.10	13.40	0.0834	2.30	0.85
	Vegetation3	42.08	27.14	0.0731	2.08	0.81	30.61	18.7	0.0821	2.27	0.79
Aug, 2012	Vegetation1	6.841	3.83	0.0968	2.63	0.87	135.53	104.71	0.0430	1.54	0.75
	Vegetation2	114.2	94.51	0.0217	1.24	0.60	55.08	39.53	0.0553	1.74	0.85
	Vegetation3	44.67	30.14	0.0534	1.71	0.81	22.06	12.77	0.0911	2.49	0.79
Sept, 2012	Vegetation1	2.991	1.54	0.1107	3.02	0.87	31.58	20.35	0.0732	2.08	0.75
	Vegetation2	132.8	96.07	0.0539	1.71	0.60	63.13	47.42	0.0477	1.61	0.85
	Vegetation3	31.63	19.62	0.0796	2.22	0.81	48.89	32.69	0.0671	1.96	0.79
Oct, 2012	Vegetation1	14.72	9.20	0.0784	2.19	0.87	24.51	14.33	0.0894	2.44	0.75
	Vegetation2	19.78	11.77	0.0865	2.38	0.60	31.21	20.41	0.0708	2.03	0.85
	Vegetation3	40.58	28.89	0.0566	1.76	0.81	59.81	42.46	0.0571	1.77	0.79
Nov, 2012	Vegetation1	15.73	10.25	0.0714	2.04	0.87	54.42	37.68	0.0612	1.84	0.75
	Vegetation2	78.97	63.18	0.0372	1.45	0.60	25.02	17.59	0.0588	1.80	0.85
	Vegetation3	17.66	10.46	0.0873	2.39	0.81	29.54	19.75	0.0671	1.96	0.79

Dec, 2012	Vegetation1	17.22	11.14	0.0726	2.07	0.87	50.75	36.12	0.0567	1.76	0.75
	Vegetation2	19.32	12.39	0.0741	2.10	0.60	36.50	25.34	0.0608	1.84	0.85
	Vegetation3	19.66	12.49	0.0755	2.13	0.81	54.91	39.45	0.0551	1.74	0.79
Jan, 2013	Vegetation1	33.69	24.93	0.0502	1.65	0.87	28.15	18.07	0.0739	2.09	0.75
	Vegetation2	26.51	17.12	0.0729	2.07	0.60	29.63	20.31	0.0629	1.88	0.85
	Vegetation3	58.43	43.11	0.0507	1.66	0.81	39.52	29.14	0.0508	1.66	0.79
Feb, 2013	Vegetation1	20.28	12.86	0.0759	2.14	0.87	76.68	55.10	0.0551	1.74	0.75
	Vegetation2	25.82	16.66	0.0730	2.08	0.60	32.87	20.79	0.0763	2.14	0.85
	Vegetation3	60.07	44.02	0.0518	1.68	0.81	31.77	21.21	0.0673	1.96	0.79
Mar, 2013	Vegetation1	16.27	10.45	0.0739	2.09	0.87	18.20	10.49	0.0918	2.50	0.75
	Vegetation2	32.33	22.25	0.0623	1.86	0.60	13.37	7.98	0.0860	2.36	0.85
	Vegetation3	30.05	20.34	0.0650	1.92	0.81	21.23	12.97	0.0821	2.27	0.79
Apr, 2013	Vegetation1	49	38.17	0.0416	1.52	0.87	57.66	37.18	0.0731	2.08	0.75
	Vegetation2	10.3	5.65	0.1001	2.72	0.60	24.55	15.67	0.0748	2.11	0.85
	Vegetation3	99.63	78.89	0.0389	1.48	0.81	64.85	47.59	0.0516	1.68	0.79

PHENOLOXIDASE

	Kraichgau						**Swabian Alb**				
	Fallow Plot						**Fallow Plot**				
Month	*Plot*	R_6	k_0	*a*	Q_{10}	*EF*	R_6	k_0	*a*	Q_{10}	*EF*
Apr, 2012	fallow1			-0.015	0.86	0.013			-0.013	0.88	0.15
	fallow2			0.009	1.1	0.005			-0.061	0.55	0.19
	fallow3			-0.044	0.65	0.015			0.003	1.03	0.12
May, 2012	fallow1			0.027	1.31	0.013			0.034	1.41	0.15
	fallow2			0.046	1.58	0.005			0.028	1.32	0.19
	fallow3			0.023	1.25	0.015			0.066	1.94	0.12
June, 2012	fallow1			-0.043	0.65	0.013			-0.137	0.25	0.15
	fallow2			-0.137	0.25	0.005			0.044	1.55	0.19
	fallow3			-0.031	0.73	0.015			0.068	1.98	0.12
July, 2012	fallow1			0	1	0.013			0.010	1.10	0.15
	fallow2			0	1	0.005			0.028	1.32	0.19
	fallow3			-0.018	0.83	0.015			0.022	1.25	0.12
Aug, 2012	fallow1			0.001	1.01	0.013			0.004	1.04	0.15
	fallow2			-0.01	0.9	0.005			0.042	1.52	0.19
	fallow3			0.02	1.22	0.015			0.009	1.09	0.12
Sept, 2012	fallow1			0.035	1.42	0.013			0.034	1.40	0.15
	fallow2			0.016	1.17	0.005			0.001	1.01	0.19
	fallow3			0.032	1.38	0.015			-0.031	0.74	0.12
Oct, 2012	fallow1			0.022	1.25	0.013			0.034	1.41	0.15
	fallow2			0.029	1.33	0.005			0.046	1.58	0.19

	fallow3	0.025	1.28	0.015	0.027	1.31	0.12
Nov, 2012	fallow1	0.003	1.03	0.013	-0.002	0.98	0.15
	fallow2	0	1	0.005	0.017	1.18	0.19
	fallow3	0.033	1.39	0.015	0.034	1.41	0.12
Dec, 2012	fallow1	-0.014	0.87	0.013	0.013	1.14	0.15
	fallow2	-0.002	0.98	0.005	0.043	1.53	0.19
	fallow3	0.003	1.03	0.015	0.009	1.10	0.12
Jan, 2013	fallow1	0.015	1.17	0.013	0.034	1.41	0.15
	fallow2	-0.023	0.79	0.005	0.023	1.26	0.19
	fallow3	0.004	1.04	0.015	0.008	1.09	0.12
Feb, 2013	fallow1	0.014	1.15	0.013	0.080	2.22	0.15
	fallow2	0.018	1.19	0.005	0.065	1.92	0.19
	fallow3	-0.006	0.94	0.015	0.038	1.46	0.12
Mar, 2013	fallow1	-0.004	0.96	0.013	0.072	2.05	0.15
	fallow2	0.019	1.2	0.005	0.036	1.43	0.19
	fallow3	0.038	1.46	0.015	0.007	1.08	0.12
Apr, 2013	fallow1	0.02	1.22	0.013	0.003	1.03	0.15
	fallow2	0.009	1.09	0.005	0.019	1.21	0.19
	fallow3	0.016	1.17	0.015	0.008	1.09	0.12

	Kraichgau					**Swabian Alb**				
	Vegetation Plot					**Vegetation Plot**				
	Plot	R_6	k_0	*a*	Q_{10}	*EF*	R_6	k_0	*a*	Q_{10}	*EF*
Apr, 2012	Vegetation1			0.031	1.36	0.025			-0.031	0.74	0.16
	Vegetation2			-0.001	0.99	0.002			-0.009	0.92	0.18
	Vegetation3			0.07	2.01	0.035			0.005	1.06	0.13
May, 2012	Vegetation1			0.057	1.76	0.025			0.027	1.31	0.16
	Vegetation2			-0.014	0.87	0.002			0.004	1.04	0.18
	Vegetation3			0.02	1.22	0.035			0.071	2.04	0.13
June, 2012	Vegetation1			0.022	1.24	0.025			0.002	1.02	0.16
	Vegetation2			-0.061	0.54	0.002			0.005	1.05	0.18
	Vegetation3			-0.014	0.87	0.035			-0.017	0.85	0.13
July, 2012	Vegetation1			-0.014	0.87	0.025			0.048	1.61	0.16
	Vegetation2			-0.007	0.93	0.002			0.041	1.51	0.18
	Vegetation3			-0.011	0.9	0.035			-0.01	0.91	0.13
Aug, 2012	Vegetation1			-0.029	0.75	0.025			-0.002	0.98	0.16
	Vegetation2			-0.012	0.89	0.002			0.028	1.33	0.18
	Vegetation3			-0.013	0.88	0.035			0.019	1.21	0.13

Sept, 2012	Vegetation1	0.021	1.23	0.025	0.039	1.48	0.16
	Vegetation2	0.005	1.06	0.002	0.023	1.26	0.18
	Vegetation3	0.035	1.42	0.035	-0.004	0.96	0.13
Oct, 2012	Vegetation1	0.017	1.18	0.025	0.027	1.31	0.16
	Vegetation2	0.02	1.22	0.002	0.023	1.26	0.18
	Vegetation3	0.011	1.12	0.035	0.015	1.16	0.13
Nov, 2012	Vegetation1	0.007	1.07	0.025	0.022	1.24	0.16
	Vegetation2	0.018	1.19	0.002	0.049	1.64	0.18
	Vegetation3	0.105	2.86	0.035	0.049	1.63	0.13
Dec, 2012	Vegetation1	-0.04	0.67	0.025	-0.012	0.88	0.16
	Vegetation2	-0.006	0.94	0.002	-0.008	0.92	0.18
	Vegetation3	0.03	1.35	0.035	0.021	1.24	0.13
Jan, 2013	Vegetation1	-0.017	0.84	0.025	0.035	1.42	0.16
	Vegetation2	-0.017	0.84	0.002	0.027	1.31	0.18
	Vegetation3	-0.012	0.89	0.035	0	1	0.13
Feb, 2013	Vegetation1	0.037	1.45	0.025	0.073	2.07	0.16
	Vegetation2	0.037	1.45	0.002	0.116	3.18	0.18
	Vegetation3	0.027	1.31	0.035	0.033	1.39	0.13
Mar, 2013	Vegetation1	0.028	1.33	0.025	0.15	4.48	0.16
	Vegetation2	0.036	1.43	0.002	0.027	1.31	0.18
	Vegetation3	0	1	0.035	0.023	1.26	0.13
Apr, 2013	Vegetation1	0	1	0.025	0.023	1.25	0.16
	Vegetation2	0.025	1.28	0.002	0.023	1.26	0.18
	Vegetation3	0.015	1.16	0.035	0.012	1.13	0.13

Appendix – II

Supplementary data – Chapter 6

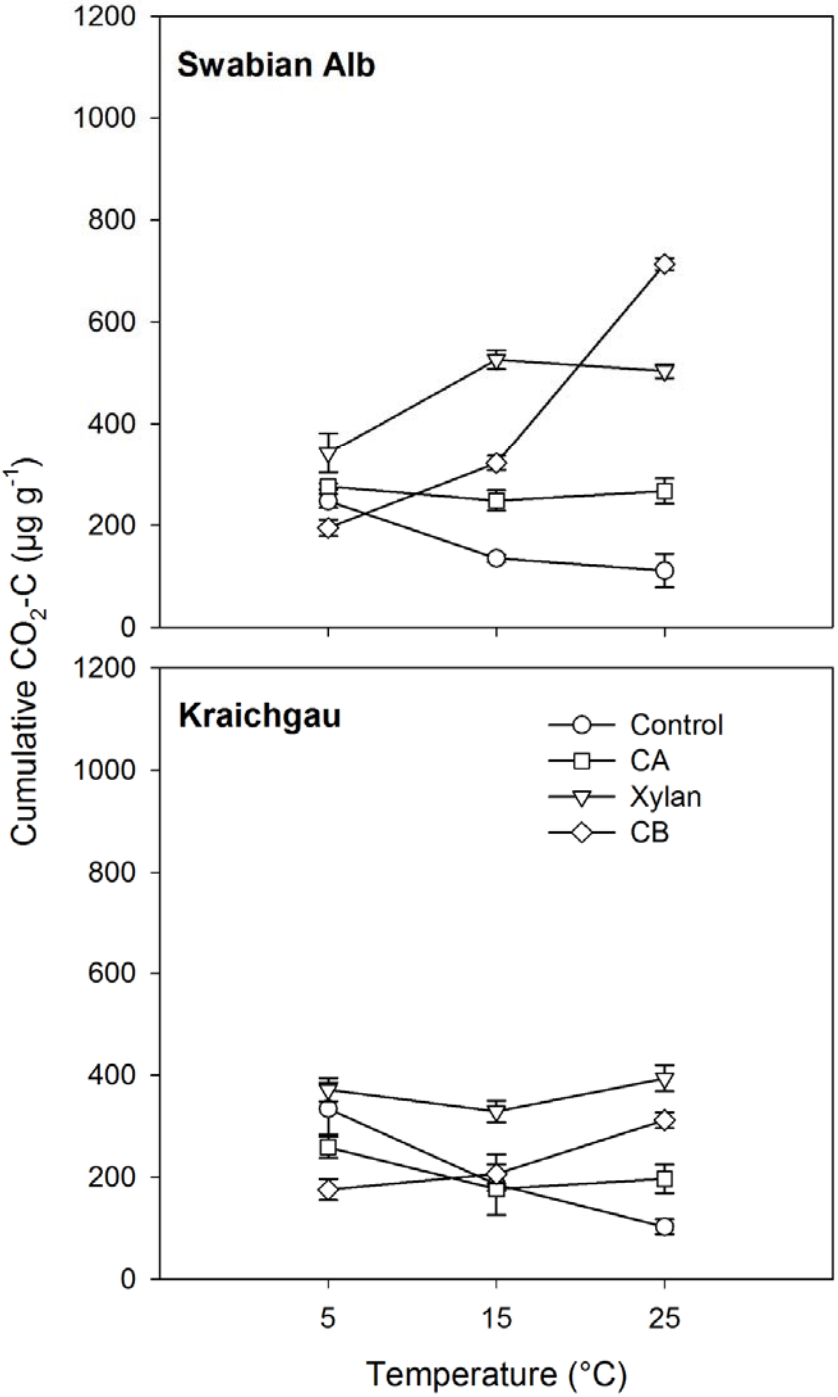

Fig. S6.1 Microbial respiration fluxes (cumulative CO_2-C) measured from bare fallow soils of both regions and different C substrate amendments.

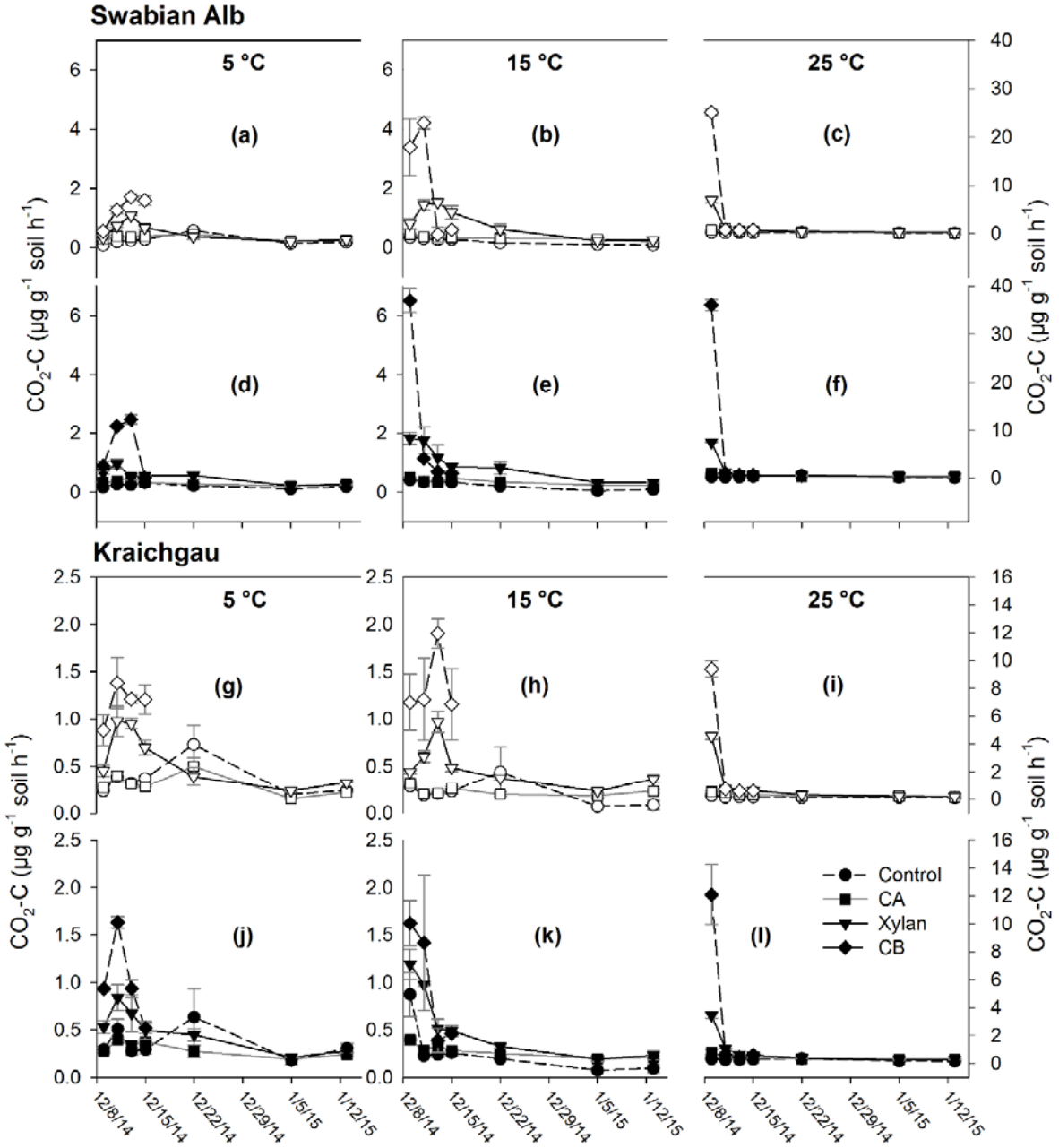

Fig. S6.2 Decomposition curves of CO_2-C fluxes measured under different C substrate amendments. Panels (a), (b), (c) & (g), (h), (i) represent bare fallow soils of the Swabian Alb and the Kraichgau, respectively. Panels (d), (e), (f) & (j), (k), (l) represent vegetated soils of the Swabian Alb and the Kraichgau, respectively. Bars indicate standard error (n = 3). Note different scales of the Swabian Alb and the Kraichgau.

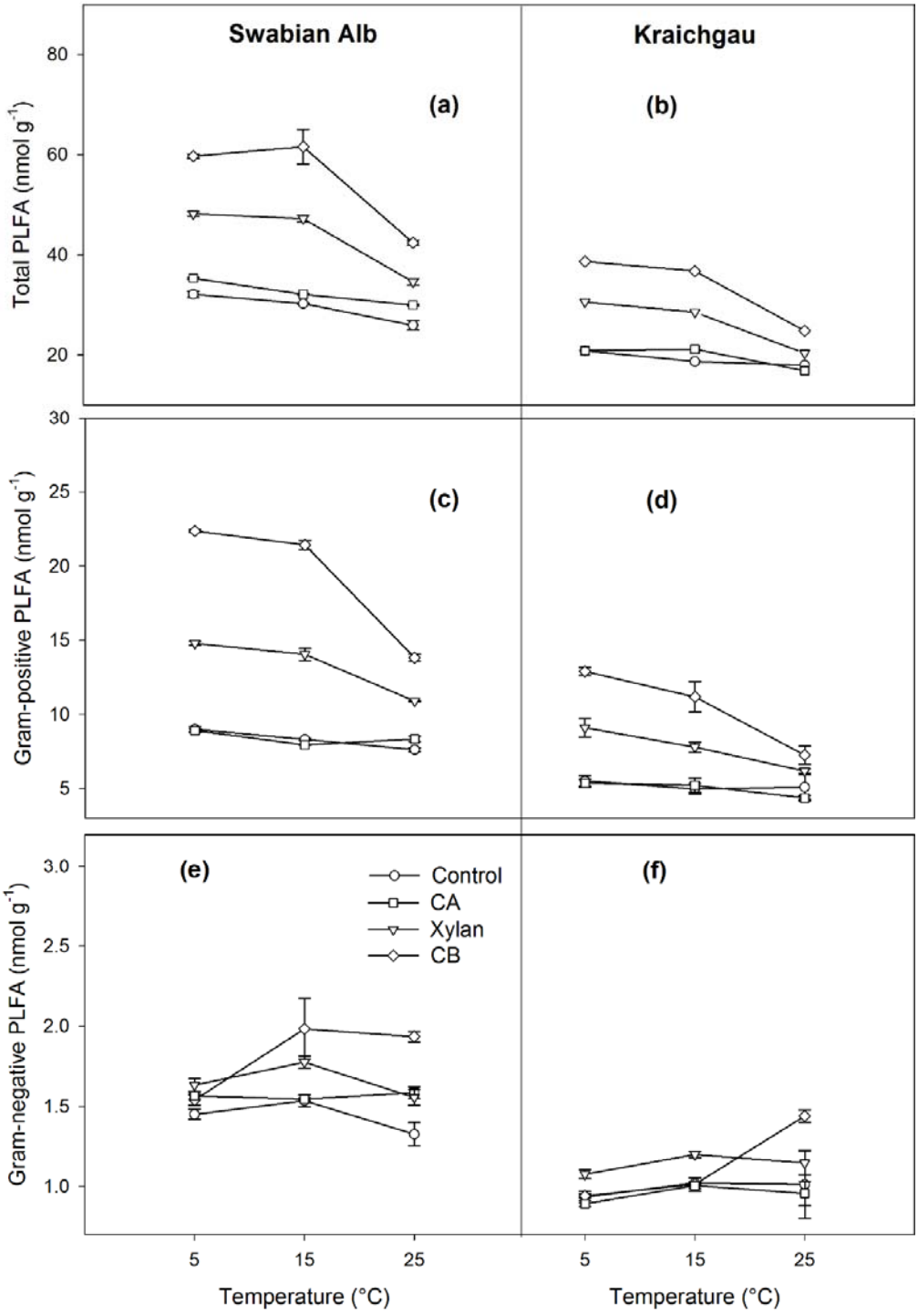

Fig. S6.3 Shifts in total, gram-positive, and gram-negative PLFAs of bare fallow soils in response to incubation temperature and availability of C substrate of varying qualities. Left panels represent PLFAs of the Swabian Alb (a, c, e) and right panels represent PLFAs of the Kraichgau (b, d, f). Bars indicate the standard error (n = 3).

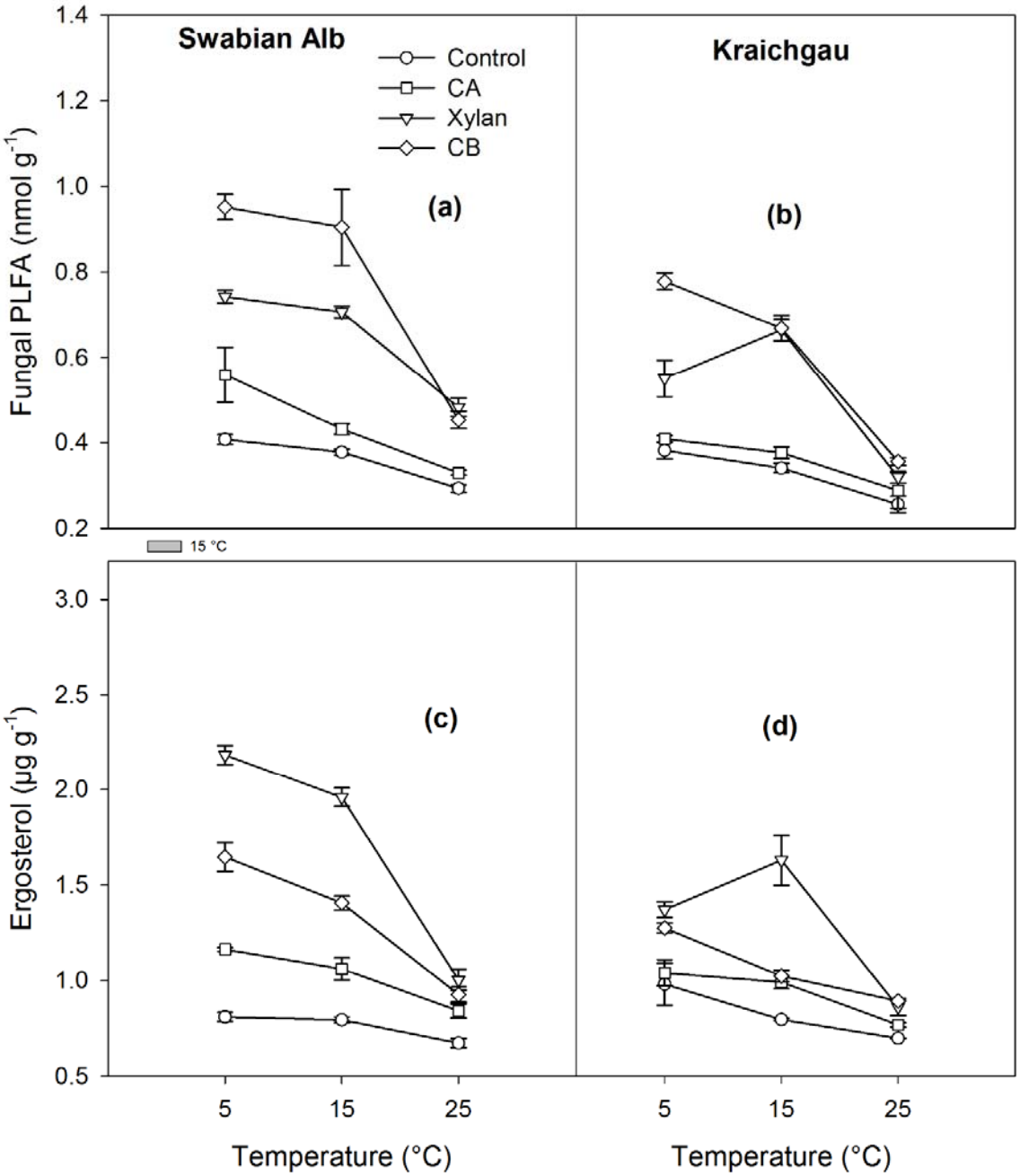

Fig. S6.4 Fungal PLFAs and ergosterol content measured in bare fallow soils of the Swabian Alb (a, c) and the Kraichgau (b, d) un-amended and amended with C substrates of varying qualities after incubation at three different temperatures. Bars indicate standard error (n = 3).

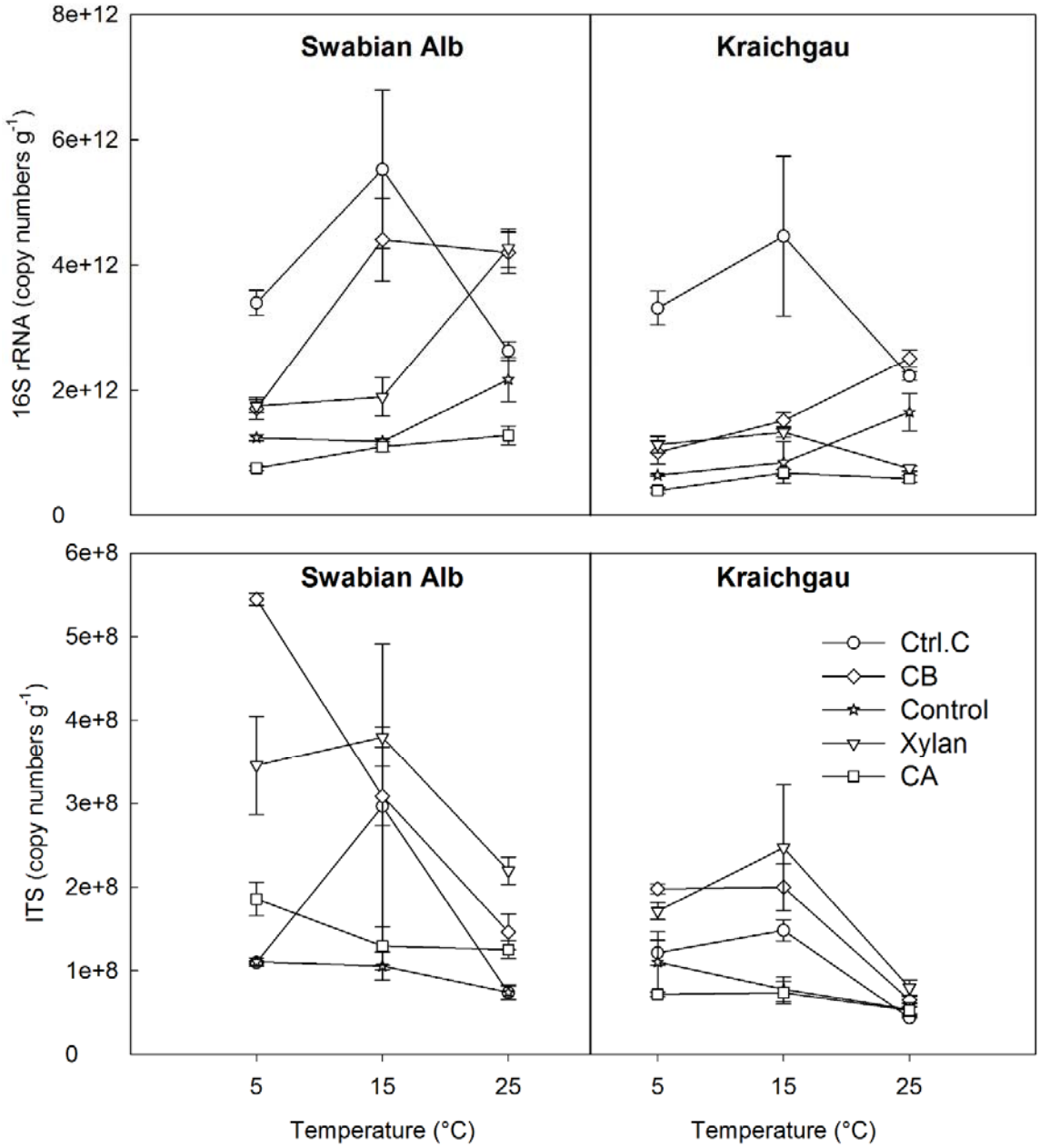

Fig. S6.5 16S rRNA gene abundance and fungal ITS fragment amounts from the Swabian Alb and the Kraichgau bare fallow soils as affected by temperature and different C substrate qualities. Bars indicate standard error (n = 3). Ctrl.C = un-amended controls after 7-day incubation and "control" after 36-day incubation.

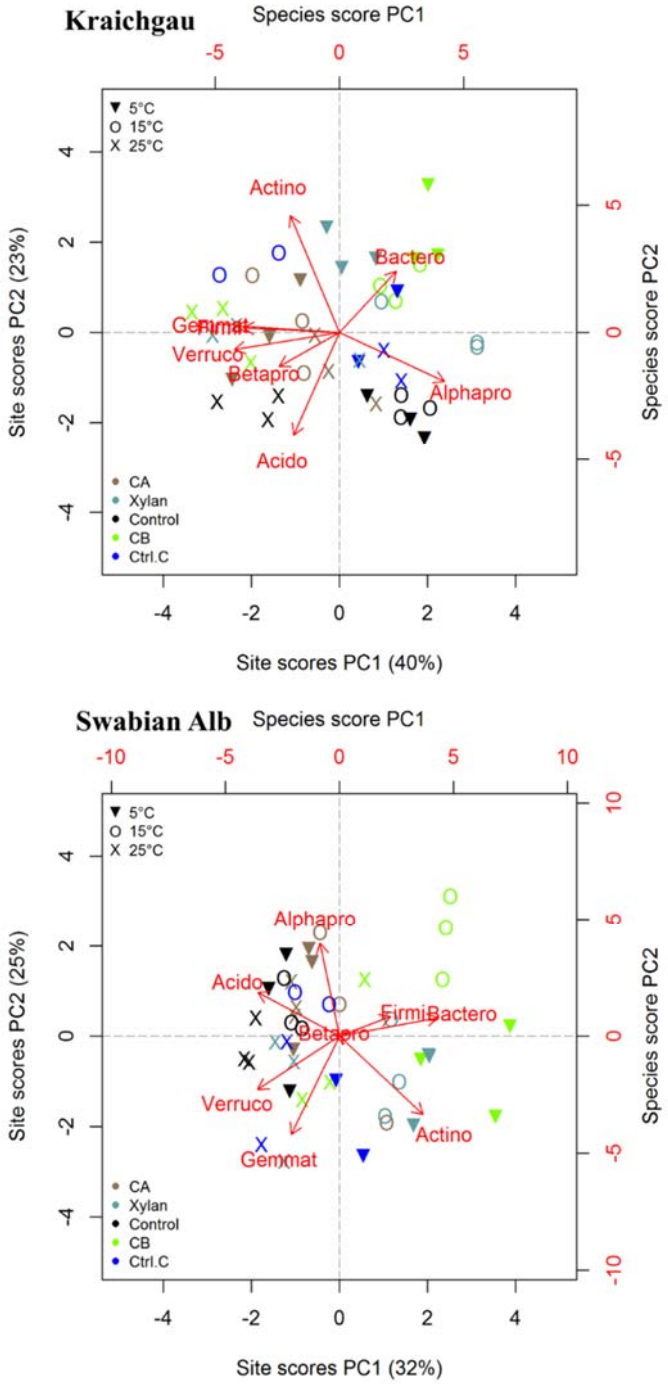

Fig. S6.6 Results of the PCA analysis from the bare fallow soils for bacterial community composition as affected by different temperatures and substrate amendments. Species scores are shown as arrows associated with different bacterial taxa (Actino = *Actinobacteria*, Alphapro = *Alphaproteobacteria*, Betapro = *Betaproteobacteria*, Acido = *Acidobacteria*, Bactero = *Bacteroidetes*, Gemmat = *Gemmatimonadetes*, Firmi = *Firmicutes*, Verruco = *Verrucomicrobia*). For legend, see Fig. 6.4.

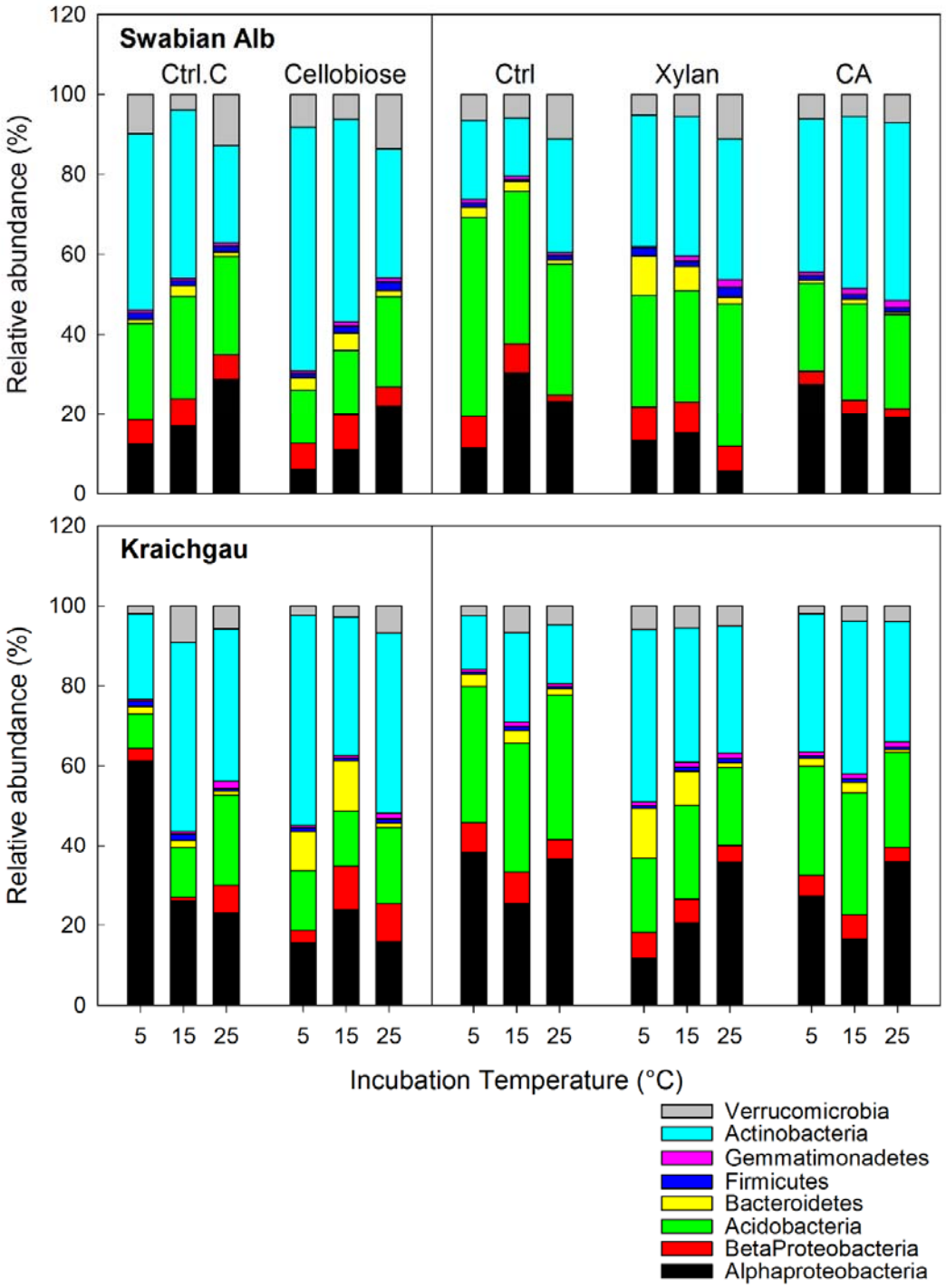

Fig. S6.7 Relative abundances (%) of the measured bacterial community members under the influence of different C substrates in vegetated soils of the Swabian Alb and the Kraichgau after exposure to different temperatures.

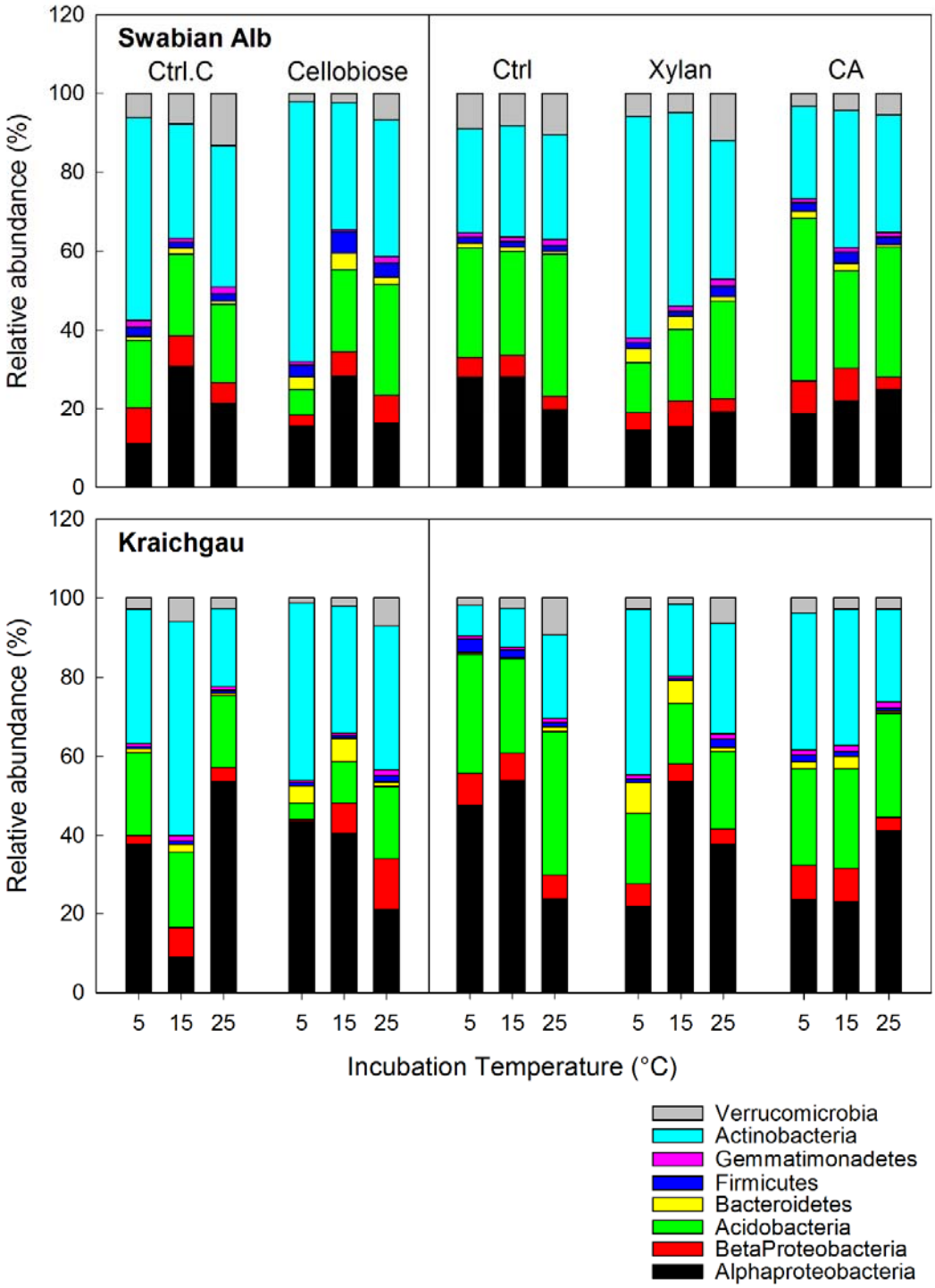

Fig. S6.8 Relative abundances (%) of the measured bacterial community members under the influence of different C substrates in bare fallow soils of the Swabian Alb and the Kraichgau after exposure to different temperatures.

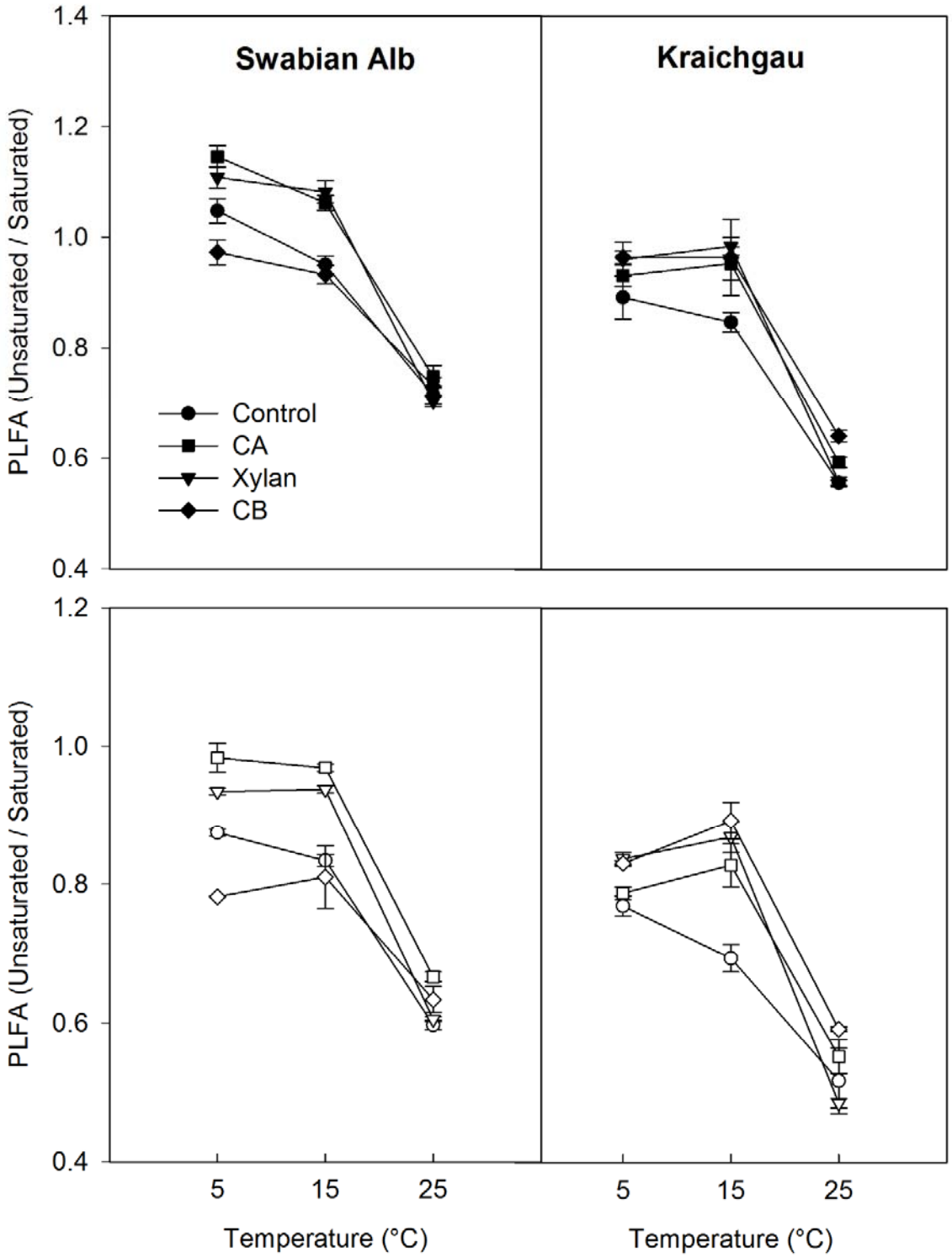

Fig. S6.9 The effect of incubation temperature on the ratio of unsaturated to saturated microbial PLFAs when amended with different substrates. The filled symbols represent vegetated soils and empty symbols the bare fallow soils. Bars indicate standard error (n = 3). Control are the samples after 36 days' incubation.

Table S6.1 Soil properties measured from the two land-use types of the Kraichgau and the Swabian Alb regions.

Region	Land-use	Basic soil properties							
		SOC mg g^{-1}	C:N	NH_4^+ µg g^{-1}	NO_3^- µg g^{-1}	Carbonate (%)	Sand (%)	Silt (%)	Clay (%)
Kraichgau	Bare fallow	7.40	9.00	2.87	4.06	n.d.	n.d.	n.d.	n.d.
	Vegetated	8.30	9.75	2.02	13.99	0.9	2.73	78.25	19.02
Swabian Alb	Bare fallow	13.90	8.86	5.49	12.83	n.d.	n.d.	n.d.	n.d.
	Vegetated	16.00	8.99	10.58	20.97	2.2	7.83	50.58	41.59

SOC = soil organic carbon, n.d. = not determined

Table S6.2 Primers, sequences, and conditions for qPCR of the respective target groups.

Target group	Primer	Sequence (5´ 3´)[a]	qPCR conditions[b]	Reference
16S rRNA genes	341F 515R	CCT ACG GGA GGC AGC AG ATT ACC GCG GCT GCT GGC A	600s at 95 °C Cycle (35): 15s at 95 °C, 30s at 60 °C, 30s at 72 °C, 30s at 75 °C (m.o.f.)	(López-Gutiérrez et al., 2004)
Fungal ITS fragment	ITS 3F ITS4R	GCA TCG ATG AAG AAC GCA GC TCC TCC GCT TAT TGA TAT GC	600s at 95 °C Cycle (35): 15s at 95 °C, 30s at 55 °C, 30s at 72 °C, 30s at 76 °C (m.o.f.)	(White et al., 1990; Manerkar et al., 2008)
Alphaproteobacteria	Eub338 Alfa685	ACT CCT ACG GGA GGC AGC AG TCT ACG RAT TTC ACC YCT AC	600s at 95 °C Cycle (35): 15s at 95 °C, 30s at 55 °C, 30s at 72 °C, 30s at 77 °C (m.o.f.)	(Fierer et al., 2005)
BetaProteobacteria	Eub338 Bet680	ACT CCT ACG GGA GGC AGC AG TCA CTG CTA CAC GYG	600s at 95 °C Cycle (35): 15s at 95 °C, 30s at 55 °C, 30s at 72 °C, 30s at 80 °C (m.o.f.)	(Fierer et al., 2005)
Acidobacteria	Acid31 Eub518	GAT CCT GGC TCA GAA TC ATT ACC GCG GCT GCT GG	600s at 95 °C Cycle (35): 15s at 95 °C, 30s at 55 °C, 30s at 72 °C, 30s at 81 °C (m.o.f.)	(Fierer et al., 2005)
Firmicutes	Lgc353 Eub518	GCA GTA GGG AAT CTT CCG ATT ACC GCG GCT GCT GG	600s at 95 °C Cycle (35): 15s at 95 °C, 30s at 60 °C, 30s at 72 °C, 30s at 79 °C (m.o.f.)	(Fierer et al., 2005)
Gemmatimonadetes	Gem440 Eub518	TTC GGR KTG TAA ACC ACT GT ATT ACC GCG GCT GCT GG	600s at 95 °C Cycle (35): 15s at 95 °C, 30s at 58 °C, 30s at 72 °C, 30s at 78 °C (m.o.f.)	(Philippot et al., 2009)
Verrucomicrobia	Verr349 Eub518	GYG GCA SCA GKC GMG AAW ATT ACC GCG GCT GCT GG	600s at 95 °C Cycle (35): 15s at 95 °C, 30s at 60 °C, 30s at 72 °C, 30s at 77 °C (m.o.f.)	(Philippot et al., 2009)
Actinobacteria	Act920F Act1200R	TAC GGC CGC AAG GCT A TCR TCC CCA CCT TCC TCC G	600s at 95 °C Cycle (35): 15s at 95 °C, 30s at 61.5 °C, 30s at 72 °C, 30s at 78 °C (m.o.f.)	(Bacchetti De Gregoris et al., 2011)
Bacteroidetes	Cfb798F Cfb967R	CRA ACA GGA TTA GAT ACC CT GGT AAG GTT CCT CGC GTA T	600s at 95 °C Cycle (35): 15s at 95 °C, 30s at 45.7 °C, 30s at 72 °C, 30s at 75 °C (m.o.f.)	(Bacchetti De Gregoris et al., 2011)

[a] M (A+C); R (A+G); W(A+T); S (G+C); Y(C+T); K(G+T)
[b] m.o.f., represents measurement of fluorescence

Table S6.3 Biomass response of bacterial taxa (absolute abundance copy numbers / g dry soil) to variations of temperature and substrate quality, from soils of two regions and land-uses. CA = conifery alcohol, CB = cellobiose, control = un-amended soils after 36 days' incubation, Ctrl.C = un-amended soils after 7 days' incubation (n = 3). Std.Er = standard error

Region	Land-use	Temperature (°C)	Substrate	*Alphaproteobacteria*		*Betaproteobacteria*		*Acidobacteria*		*Bacteroidetes*	
				Average	Std. Er	Average	Std. Er	Average	Std. Er	Average	Std. Er
Kraichgau	Bare fallow	5	CA	9.17E+08	2.21E+08	2.67E+08	3.59E+07	9.12E+08	1.47E+08	6.04E+07	3.12E+06
		15	CA	1.35E+09	6.98E+08	4.58E+08	9.78E+07	1.37E+09	2.19E+08	1.63E+08	1.66E+07
		25	CA	2.82E+09	1.15E+08	2.32E+08	1.92E+07	1.81E+09	7.00E+07	4.14E+07	2.72E+06
		5	xylan	1.67E+09	2.24E+08	4.44E+08	3.69E+07	1.39E+09	1.82E+08	6.02E+08	6.42E+07
		15	xylan	5.98E+09	1.07E+09	4.82E+08	6.81E+07	1.68E+09	7.01E+07	6.36E+08	4.44E+07
		25	xylan	4.31E+09	1.28E+09	3.88E+08	6.34E+07	2.12E+09	1.00E+08	1.09E+08	5.79E+05
		5	control	2.09E+09	2.42E+08	3.60E+08	9.55E+07	1.32E+09	1.33E+08	1.48E+08	1.81E+07
		15	control	4.82E+09	7.38E+08	6.11E+08	4.86E+07	2.13E+09	1.21E+08	1.67E+08	6.59E+06
		25	control	1.34E+09	2.17E+08	3.40E+08	8.54E+07	2.06E+09	3.32E+08	5.69E+07	1.35E+07
		5	CB	4.21E+09	1.06E+09	5.97E+07	5.03E+07	4.76E+08	2.38E+08	3.78E+08	1.10E+07
		15	CB	4.39E+09	8.61E+08	9.18E+08	3.74E+08	1.08E+09	8.47E+07	5.84E+08	3.98E+07
		25	CB	2.17E+09	4.33E+08	1.33E+09	2.73E+08	1.76E+09	1.63E+08	1.05E+08	1.67E+07
		5	Ctrl.C	7.21E+09	1.73E+09	4.11E+08	3.17E+07	3.71E+09	1.38E+09	1.97E+08	1.34E+07
		15	Ctrl.C	1.38E+09	3.32E+08	1.12E+09	5.17E+06	2.87E+09	2.39E+08	3.11E+08	1.98E+07
		25	Ctrl.C	3.73E+09	3.05E+08	2.49E+08	3.19E+07	1.25E+09	3.18E+08	5.02E+07	7.80E+06
Kraichgau	Vegetated	5	CA	2.13E+09	1.84E+08	3.99E+08	3.35E+07	2.11E+09	6.96E+07	1.40E+08	1.59E+07
		15	CA	2.26E+09	3.51E+08	8.20E+08	9.81E+07	4.19E+09	4.97E+08	3.59E+08	2.48E+07
		25	CA	5.06E+09	7.04E+08	5.13E+08	5.30E+06	3.29E+09	2.08E+08	9.88E+07	7.83E+06
		5	xylan	1.04E+09	2.30E+08	5.42E+08	6.39E+07	1.66E+09	5.30E+08	1.03E+09	1.75E+08
		15	xylan	2.29E+09	7.42E+08	6.98E+08	2.24E+08	2.86E+09	9.17E+08	9.18E+08	1.48E+08
		25	xylan	5.19E+09	1.94E+09	5.35E+08	7.71E+07	2.47E+09	3.30E+08	1.52E+08	1.08E+07
		5	control	2.60E+09	1.94E+08	5.22E+08	7.60E+07	2.27E+09	3.41E+08	2.15E+08	3.72E+07
		15	control	2.52E+09	1.26E+08	7.84E+08	1.15E+08	3.30E+09	4.22E+08	3.03E+08	4.85E+07
		25	control	2.18E+09	4.08E+08	1.77E+08	9.89E+07	2.15E+09	2.87E+08	8.74E+07	1.17E+07
		5	CB	2.17E+09	6.25E+08	5.22E+08	2.53E+08	2.02E+09	1.78E+08	1.35E+09	2.16E+08
		15	CB	5.87E+09	6.42E+08	2.73E+09	4.30E+08	3.46E+09	2.33E+08	3.27E+09	1.11E+09
		25	CB	3.01E+09	2.15E+08	1.85E+09	2.92E+08	3.71E+09	2.18E+08	1.97E+08	3.48E+07
		5	Ctrl.C	4.81E+09	2.07E+09	2.02E+08	5.08E+06	7.13E+08	3.84E+08	1.15E+08	8.88E+06
		15	Ctrl.C	2.64E+09	3.69E+08	9.91E+07	4.14E+07	1.46E+09	7.87E+08	2.04E+08	1.05E+08
		25	Ctrl.C	3.31E+09	1.35E+08	9.87E+08	5.51E+07	3.26E+09	3.69E+07	1.36E+08	1.43E+07

Region	Land-use	Temperature (°C)	Substrate	*Firmicutes*		*Gemmatimonadetes*		*Actinobacteria*		*Verrucomicrobia*	
				Average	Std. Er	Average	Std. Er	Average	Std. Er	Average	Std. Er
Kraichgau	Bare fallow	5	CA	5.73E+07	3.49E+06	5.00E+07	1.24E+07	1.50E+09	6.30E+08	1.49E+08	4.45E+07
		15	CA	6.84E+07	2.71E+07	9.42E+07	2.91E+07	2.15E+09	7.98E+08	1.63E+08	4.78E+07
		25	CA	5.65E+07	7.95E+06	9.99E+07	2.46E+07	1.73E+09	5.52E+08	2.06E+08	6.63E+07
		5	xylan	7.32E+07	1.28E+07	8.01E+07	2.38E+07	3.57E+09	1.29E+09	2.33E+08	7.43E+07
		15	xylan	5.62E+07	8.02E+06	6.20E+07	2.31E+07	2.01E+09	5.97E+08	1.67E+08	5.30E+07
		25	xylan	2.17E+08	2.77E+07	1.46E+08	2.19E+07	2.98E+09	2.37E+08	6.91E+08	6.11E+07
		5	control	2.21E+07	6.37E+06	3.44E+07	1.08E+07	3.38E+08	2.12E+08	8.36E+07	3.89E+07
		15	control	3.84E+07	6.92E+06	5.48E+07	1.11E+07	8.87E+08	2.43E+08	2.28E+08	6.74E+07
		25	control	7.27E+07	1.25E+07	5.53E+07	1.18E+07	1.20E+09	1.97E+08	5.26E+08	1.87E+08
		5	CB	7.45E+07	8.08E+06	4.54E+07	2.19E+07	4.07E+09	5.27E+08	1.14E+08	8.95E+06
		15	CB	8.00E+07	6.47E+06	6.67E+07	1.76E+07	3.51E+09	7.97E+08	1.71E+08	5.43E+07
		25	CB	1.52E+08	2.60E+07	1.64E+08	5.30E+07	3.83E+09	9.74E+08	7.15E+08	1.06E+08
		5	Ctrl.C	9.08E+07	4.67E+06	1.32E+08	3.15E+06	6.53E+09	1.81E+09	5.25E+08	8.78E+06
		15	Ctrl.C	1.23E+08	1.70E+07	2.10E+08	1.10E+07	8.31E+09	1.84E+09	8.85E+08	1.40E+07
		25	Ctrl.C	5.05E+07	7.64E+06	2.12E+07	NA	1.38E+09	1.05E+08	1.84E+08	1.54E+06
Kraichgau	Vegetated	5	CA	6.30E+07	1.30E+07	7.75E+07	1.53E+07	2.93E+09	9.82E+08	1.66E+08	4.40E+07
		15	CA	1.29E+08	3.16E+07	1.63E+08	3.52E+07	5.57E+09	1.49E+09	5.92E+08	2.35E+08
		25	CA	8.11E+07	4.77E+06	1.91E+08	2.29E+07	4.25E+09	7.23E+08	5.45E+08	3.24E+07
		5	xylan	6.21E+07	8.67E+06	8.42E+07	3.25E+07	3.90E+09	1.16E+09	4.82E+08	1.00E+08
		15	xylan	1.19E+08	2.85E+07	1.33E+08	2.35E+07	3.66E+09	8.51E+08	6.09E+08	2.73E+08
		25	xylan	1.25E+08	7.69E+06	1.80E+08	5.57E+07	4.03E+09	6.40E+08	6.68E+08	2.09E+08
		5	control	3.26E+07	1.56E+07	4.72E+07	2.03E+07	9.90E+08	5.81E+08	1.81E+08	9.06E+07
		15	control	1.01E+08	1.58E+07	1.40E+08	7.46E+07	2.31E+09	5.43E+08	7.52E+08	3.80E+08
		25	control	3.31E+07	1.84E+07	4.72E+07	2.36E+07	9.13E+08	2.86E+08	2.92E+08	7.16E+07
		5	CB	1.15E+08	1.60E+07	9.82E+07	2.30E+07	8.00E+09	2.42E+09	3.58E+08	1.08E+08
		15	CB	1.61E+08	1.81E+07	1.77E+08	2.82E+07	8.62E+09	8.67E+08	6.93E+08	9.73E+07
		25	CB	2.11E+08	3.68E+07	2.60E+08	4.44E+07	8.72E+09	7.79E+08	1.33E+09	1.33E+08
		5	Ctrl.C	8.30E+07	#DIV/0!	3.01E+07	1.05E+07	1.53E+09	3.23E+08	1.62E+08	7.92E+07
		15	Ctrl.C	1.63E+08	3.43E+07	9.00E+07	5.56E+07	5.67E+09	NA	1.21E+09	1.04E+09
		25	Ctrl.C	9.48E+07	1.47E+07	2.65E+08	1.63E+07	5.50E+09	6.11E+08	8.20E+08	3.74E+07

Region	Land-use	Temperature (°C)	Substrate	*Alphaproteobacteria*		*Betaproteobacteria*		*Acidobacteria*		*Bacteroidetes*	
				Average	Std. Er	Average	Std. Er	Average	Std. Er	Average	Std. Er
Swabian Alb	Bare fallow	5	CA	1.08E+09	2.62E+08	4.34E+08	9.57E+06	2.23E+09	1.89E+08	8.54E+07	1.22E+07
		15	CA	1.52E+09	6.25E+08	5.20E+08	4.44E+07	1.65E+09	5.33E+08	1.20E+08	6.40E+06
		25	CA	3.26E+09	4.29E+08	4.09E+08	7.58E+07	4.36E+09	7.33E+08	8.43E+07	1.54E+07
		5	xylan	3.25E+09	4.09E+08	1.08E+09	1.21E+08	2.98E+09	3.56E+08	8.43E+08	1.51E+07
		15	xylan	3.81E+09	7.85E+08	1.62E+09	1.79E+07	4.37E+09	7.41E+08	8.03E+08	1.06E+08
		25	xylan	5.12E+09	1.90E+09	7.79E+08	7.78E+07	6.02E+09	9.05E+08	2.59E+08	4.86E+07
		5	control	3.76E+09	1.16E+09	6.68E+08	7.19E+07	3.73E+09	3.65E+08	1.58E+08	2.62E+07
		15	control	4.13E+09	2.98E+08	8.22E+08	2.26E+07	3.87E+09	8.30E+08	1.71E+08	1.04E+07
		25	control	2.70E+09	3.75E+08	4.60E+08	3.44E+07	4.86E+09	1.16E+08	8.95E+07	1.12E+07
		5	CB	1.32E+09	4.19E+08	2.20E+08	1.61E+08	5.10E+08	4.87E+08	2.65E+08	2.59E+07
		15	CB	3.23E+09	5.16E+08	7.23E+08	1.88E+08	2.30E+09	4.83E+07	4.68E+08	9.40E+06
		25	CB	2.10E+09	6.76E+08	9.08E+08	2.08E+08	3.46E+09	2.81E+08	2.08E+08	1.56E+07
		5	Ctrl.C	1.04E+09	7.16E+07	7.62E+08	NA	1.61E+09	7.92E+07	9.38E+07	5.87E+05
		15	Ctrl.C	4.59E+09	1.43E+09	1.17E+09	3.35E+08	3.15E+09	1.23E+09	2.12E+08	7.20E+07
		25	Ctrl.C	2.94E+09	1.84E+09	6.67E+08	5.78E+07	2.52E+09	1.06E+09	1.04E+08	2.22E+07
Swabian Alb	Vegetated	5	CA	4.48E+09	3.35E+08	5.48E+08	8.33E+07	3.66E+09	5.50E+08	1.18E+08	1.38E+07
		15	CA	4.09E+09	3.30E+08	7.23E+08	5.26E+07	5.11E+09	1.20E+09	2.59E+08	9.00E+06
		25	CA	4.29E+09	3.15E+08	5.48E+08	3.39E+07	5.26E+09	1.37E+08	1.19E+08	1.39E+07
		5	xylan	2.05E+09	3.22E+08	1.30E+09	1.58E+08	4.67E+09	1.68E+09	1.45E+09	5.63E+07
		15	xylan	2.65E+09	5.62E+08	1.41E+09	4.07E+08	4.75E+09	6.80E+08	1.03E+09	1.21E+08
		25	xylan	8.25E+08	1.79E+08	8.99E+08	7.30E+07	5.09E+09	2.61E+08	2.50E+08	7.22E+06
		5	control	1.36E+09	1.14E+08	9.73E+08	1.06E+08	5.98E+09	2.18E+08	3.04E+08	2.18E+07
		15	control	4.54E+09	6.06E+08	1.06E+09	1.27E+08	5.57E+09	2.53E+08	3.60E+08	1.03E+07
		25	control	3.43E+09	2.25E+08	2.83E+08	2.56E+08	4.93E+09	7.02E+08	1.51E+08	1.49E+07
		5	CB	1.58E+09	4.50E+08	1.79E+09	2.28E+08	3.96E+09	4.52E+08	8.83E+08	9.61E+07
		15	CB	3.02E+09	2.04E+08	2.41E+09	2.60E+08	4.47E+09	4.86E+08	1.16E+09	1.23E+08
		25	CB	6.96E+09	7.03E+08	1.60E+09	5.05E+08	7.25E+09	1.07E+09	4.45E+08	1.93E+07
		5	Ctrl.C	3.05E+09	6.25E+08	1.50E+09	2.87E+08	5.99E+09	7.30E+08	2.35E+08	1.19E+06
		15	Ctrl.C	1.27E+09	2.87E+08	5.02E+08	2.78E+07	1.88E+09	9.43E+07	2.01E+08	2.11E+07
		25	Ctrl.C	6.46E+09	7.76E+08	1.35E+09	9.85E+07	5.53E+09	8.78E+08	2.54E+08	2.24E+07

Region	Land-use	Temperature (°C)	Substrate	*Firmicutes*		*Gemmatimonadetes*		*Actinobacteria*		*Verrucomicrobia*	
				Average	Std. Er	Average	Std. Er	Average	Std. Er	Average	Std. Er
Swabian Alb	Bare fallow	5	CA	1.27E+08	6.78E+07	6.85E+07	3.14E+07	1.40E+09	4.35E+08	2.11E+08	1.17E+08
		15	CA	1.80E+08	2.60E+07	7.79E+07	2.37E+07	2.18E+09	3.58E+08	2.88E+08	6.33E+07
		25	CA	2.50E+08	5.67E+06	1.47E+08	1.93E+07	3.82E+09	2.78E+08	6.82E+08	7.03E+07
		5	xylan	3.74E+08	2.68E+07	2.60E+08	4.81E+07	1.37E+10	2.88E+09	1.43E+09	3.48E+08
		15	xylan	3.00E+08	5.31E+07	3.25E+08	3.32E+07	1.20E+10	1.64E+09	1.22E+09	2.20E+08
		25	xylan	5.84E+08	1.05E+08	4.14E+08	2.56E+07	8.57E+09	9.44E+08	2.86E+09	9.06E+07
		5	control	1.98E+08	6.37E+06	1.62E+08	1.30E+07	3.51E+09	6.64E+08	1.20E+09	2.05E+08
		15	control	2.18E+08	1.72E+07	1.69E+08	1.41E+07	4.13E+09	3.68E+08	1.19E+09	8.53E+07
		25	control	2.04E+08	1.93E+07	1.94E+08	2.23E+07	3.60E+09	2.35E+08	1.41E+09	5.98E+07
		5	CB	2.53E+08	6.30E+07	7.45E+07	2.31E+07	5.71E+09	1.06E+09	2.20E+08	1.73E+08
		15	CB	6.22E+08	1.18E+08	5.32E+07	2.26E+07	3.82E+09	1.08E+09	2.59E+08	2.11E+07
		25	CB	4.45E+08	2.81E+07	2.05E+08	6.28E+07	4.60E+09	1.30E+09	8.50E+08	1.52E+08
		5	Ctrl.C	2.26E+08	2.79E+07	1.74E+08	4.84E+07	5.30E+09	2.04E+09	6.25E+08	1.65E+08
		15	Ctrl.C	2.18E+08	4.85E+06	1.45E+08	3.23E+07	4.13E+09	7.70E+08	1.21E+09	5.27E+08
		25	Ctrl.C	2.36E+08	1.29E+07	2.16E+08	5.68E+07	4.69E+09	1.43E+08	1.75E+09	1.79E+08
Swabian Alb	Vegetated	5	CA	1.89E+08	2.34E+07	1.56E+08	8.97E+06	6.37E+09	1.13E+09	9.91E+08	8.63E+07
		15	CA	2.16E+08	1.25E+07	3.08E+08	5.24E+07	8.92E+09	1.06E+09	1.16E+09	2.00E+08
		25	CA	2.63E+08	4.28E+07	4.11E+08	7.54E+07	1.03E+10	1.68E+09	1.61E+09	2.46E+08
		5	xylan	2.84E+08	4.17E+07	1.00E+08	5.83E+07	5.01E+09	9.42E+08	8.33E+08	2.36E+08
		15	xylan	2.20E+08	2.55E+07	2.13E+08	2.52E+07	5.90E+09	4.93E+08	9.48E+08	1.79E+08
		25	xylan	3.50E+08	4.89E+07	2.89E+08	7.51E+07	5.20E+09	9.58E+08	1.63E+09	3.54E+08
		5	control	1.15E+08	9.95E+06	1.11E+08	4.39E+07	2.38E+09	1.91E+08	7.87E+08	8.23E+07
		15	control	8.79E+07	3.96E+07	1.15E+08	4.25E+07	2.22E+09	4.77E+08	8.93E+08	2.28E+08
		25	control	1.84E+08	8.23E+07	1.20E+08	3.02E+07	4.29E+09	6.75E+08	1.64E+09	1.11E+08
		5	CB	3.15E+08	4.43E+07	2.13E+08	2.73E+07	2.16E+10	7.93E+09	2.53E+09	7.25E+08
		15	CB	5.08E+08	5.42E+07	2.92E+08	5.57E+07	1.41E+10	2.10E+09	1.77E+09	3.02E+08
		25	CB	7.12E+08	3.73E+07	3.10E+08	6.93E+06	1.02E+10	1.27E+09	4.23E+09	9.72E+07
		5	Ctrl.C	3.98E+08	4.79E+07	2.18E+08	8.79E+07	1.08E+10	4.30E+08	2.46E+09	7.49E+08
		15	Ctrl.C	7.99E+07	3.49E+06	4.81E+07	1.86E+07	3.11E+09	2.22E+08	2.90E+08	4.51E+07
		25	Ctrl.C	3.36E+08	3.25E+07	1.73E+08	7.39E+06	5.46E+09	5.63E+08	2.97E+09	1.03E+09

Appendix – III

Supplementary data – Chapter 7

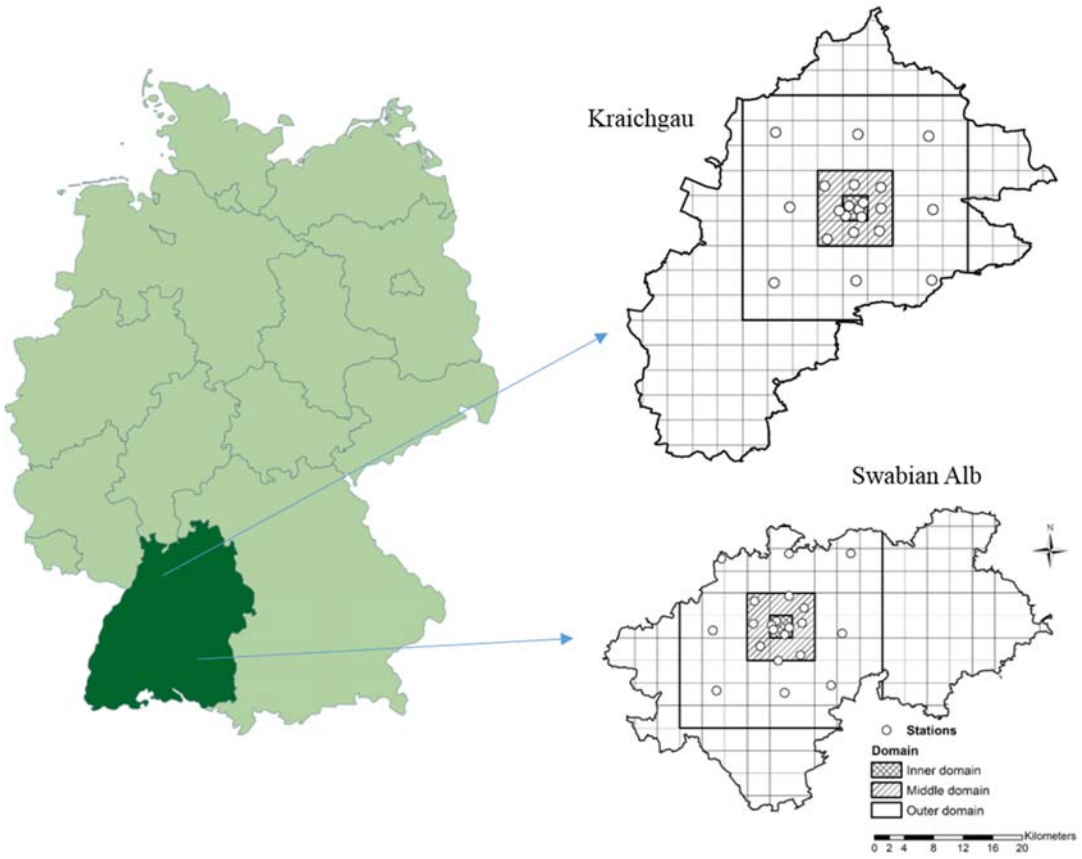

Fig. S7.1 The two investigated landscapes in southwest Germany showing the 41 moisture network sites.

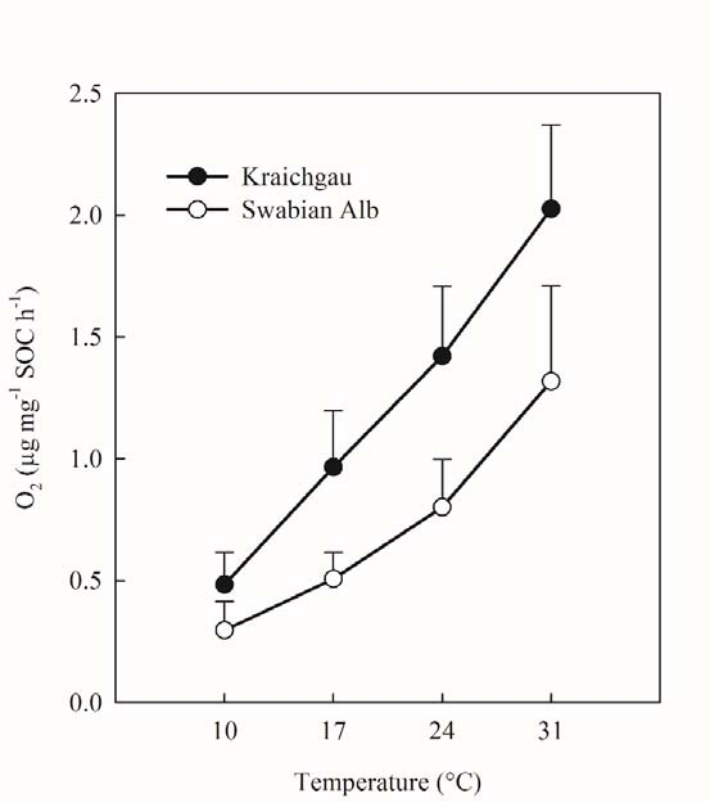

Fig. S7.2 Soil respiration normalized to SOC content, measured in terms of oxygen consumption response of soil microorganisms to increasing temperatures in the Kraichgau and the Swabian Alb. Bars indicate standard deviation for the Kraichgau (n = 21) and the Swabian Alb (n = 19).

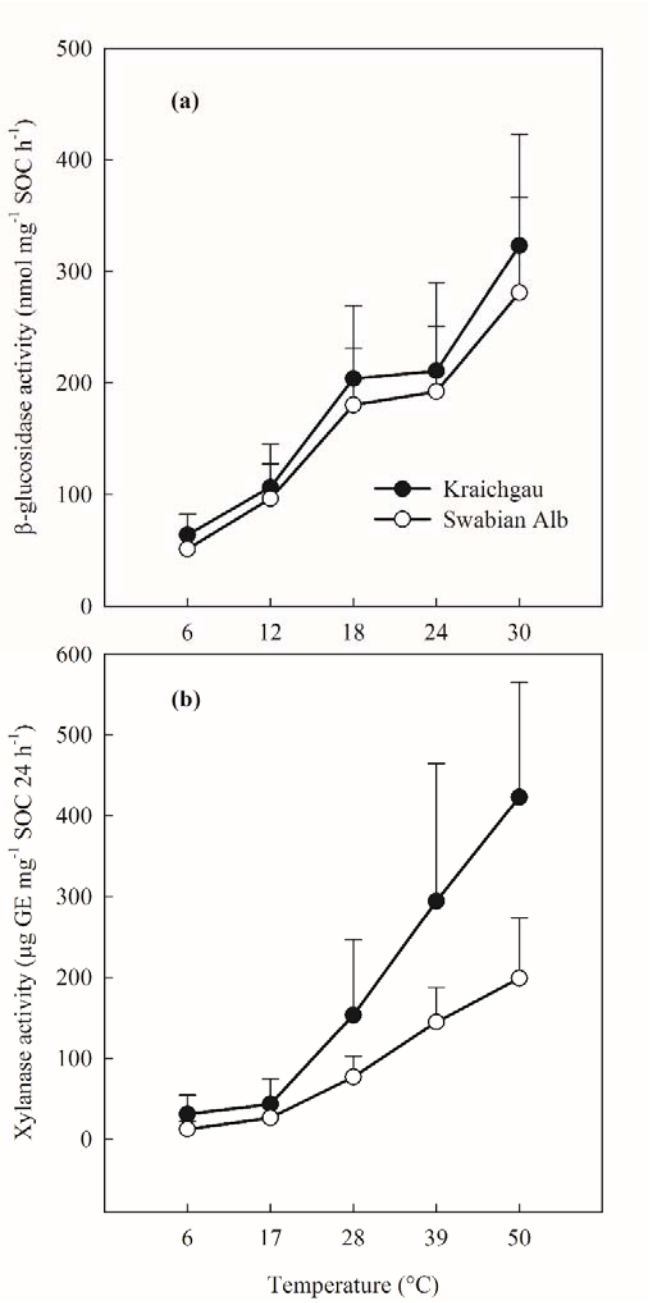

Fig. S7.3 Response of β-glucosidase (a) and xylanase (b) activities to changing temperature in the Kraichgau and the Swabian Alb. Enzyme activities are normalized to SOC per site. Bars indicate standard deviation for the Kraichgau (n = 21) and the Swabian Alb (n = 19).

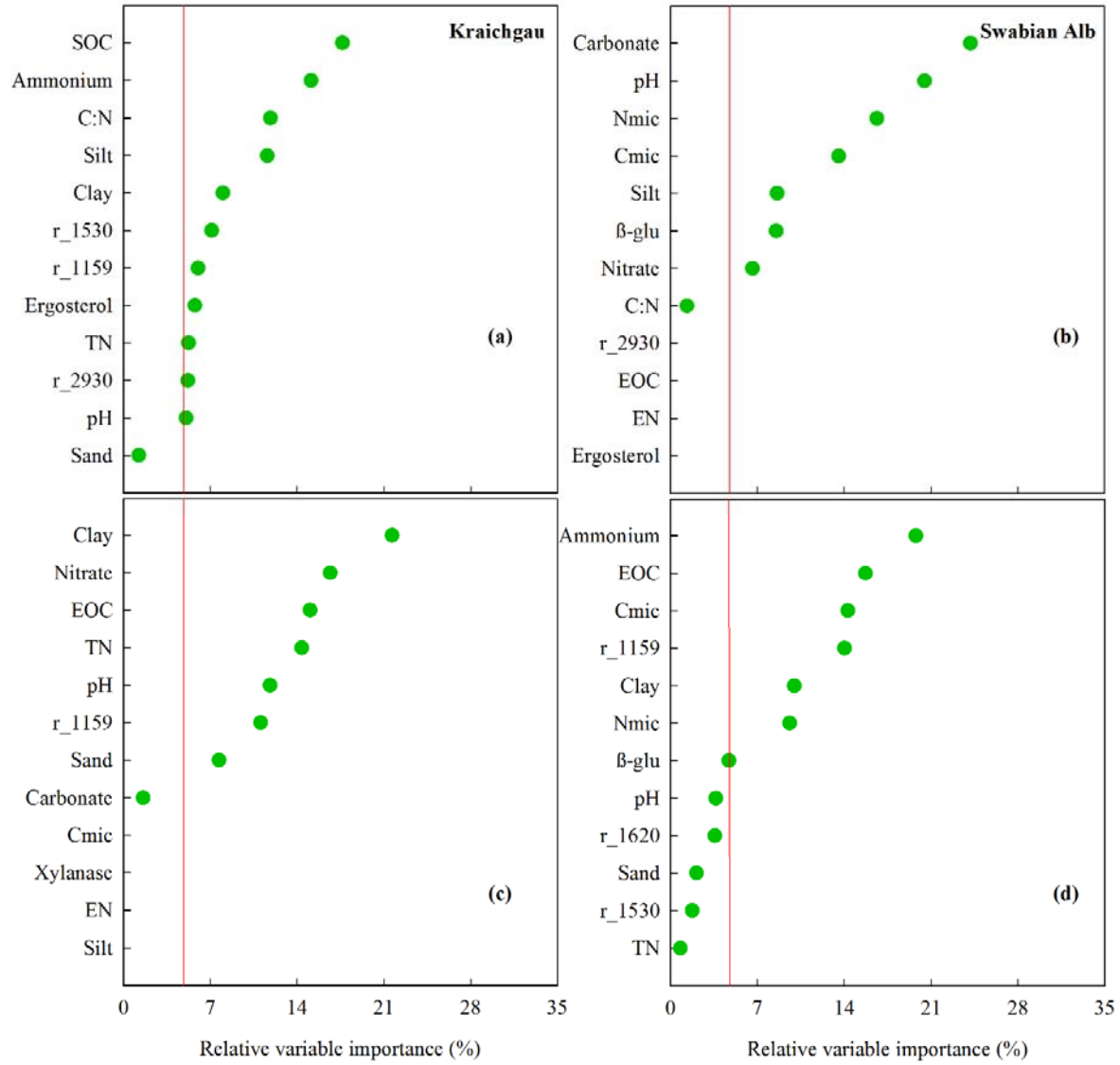

Fig. S7.4 Variable importance as given by the tree ensemble regression models for selecting most important variables explaining basal soil respiration (measured at 31 °C) and its temperature sensitivity in the Kraichgau (a & c) and the Swabian Alb (b & d), respectively. Line indicates theoretical importance when all explanatory variables have similar importance.

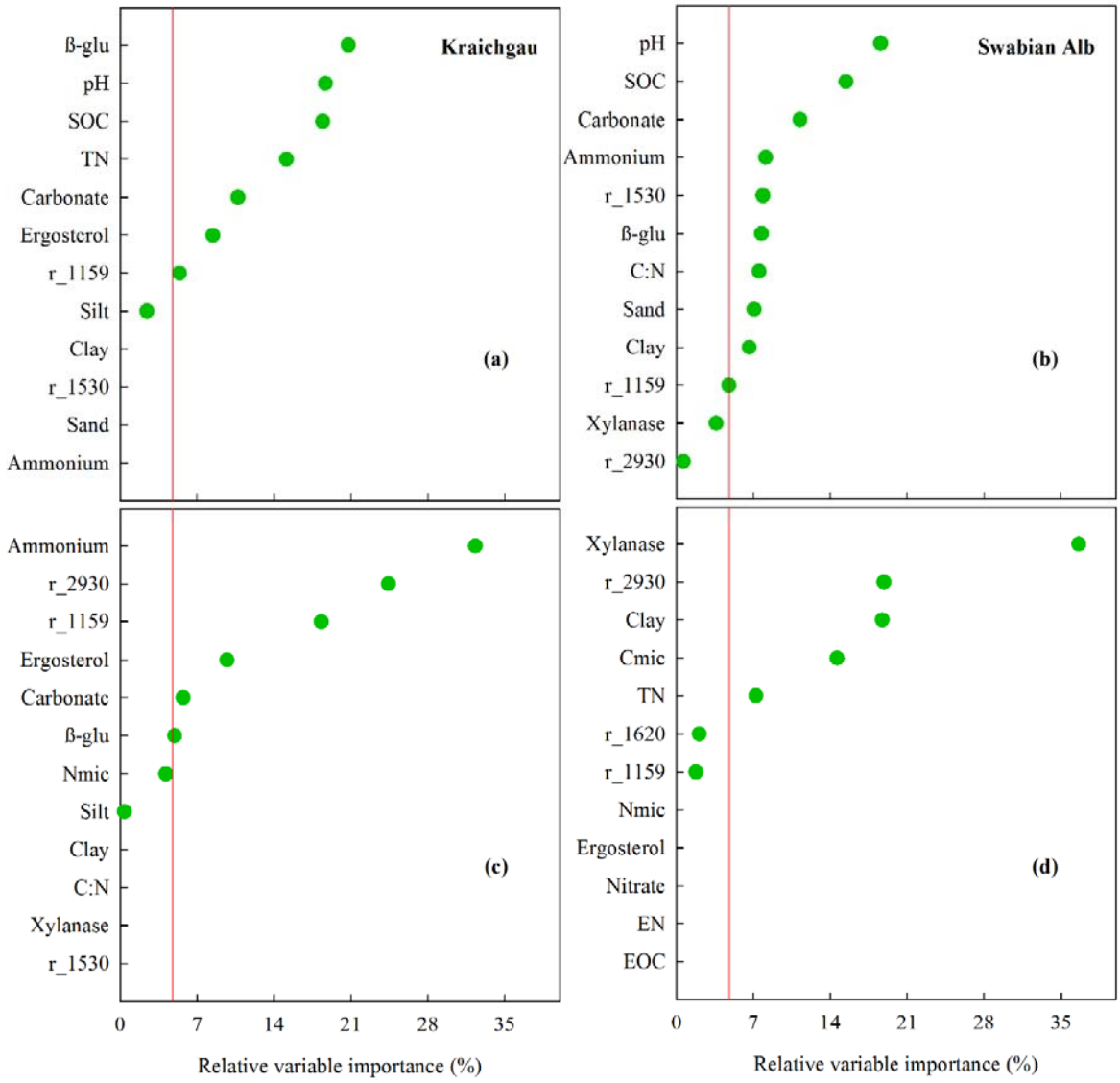

Fig. S7.5 Variable importance as given by the tree ensemble regression models for selecting most important variables explaining temperature sensitivity of β-glucosidase and xylanase activities in the Kraichgau (a & c) and the Swabian Alb (b & d), respectively. Line indicates theoretical importance when all explanatory variables have similar importance.

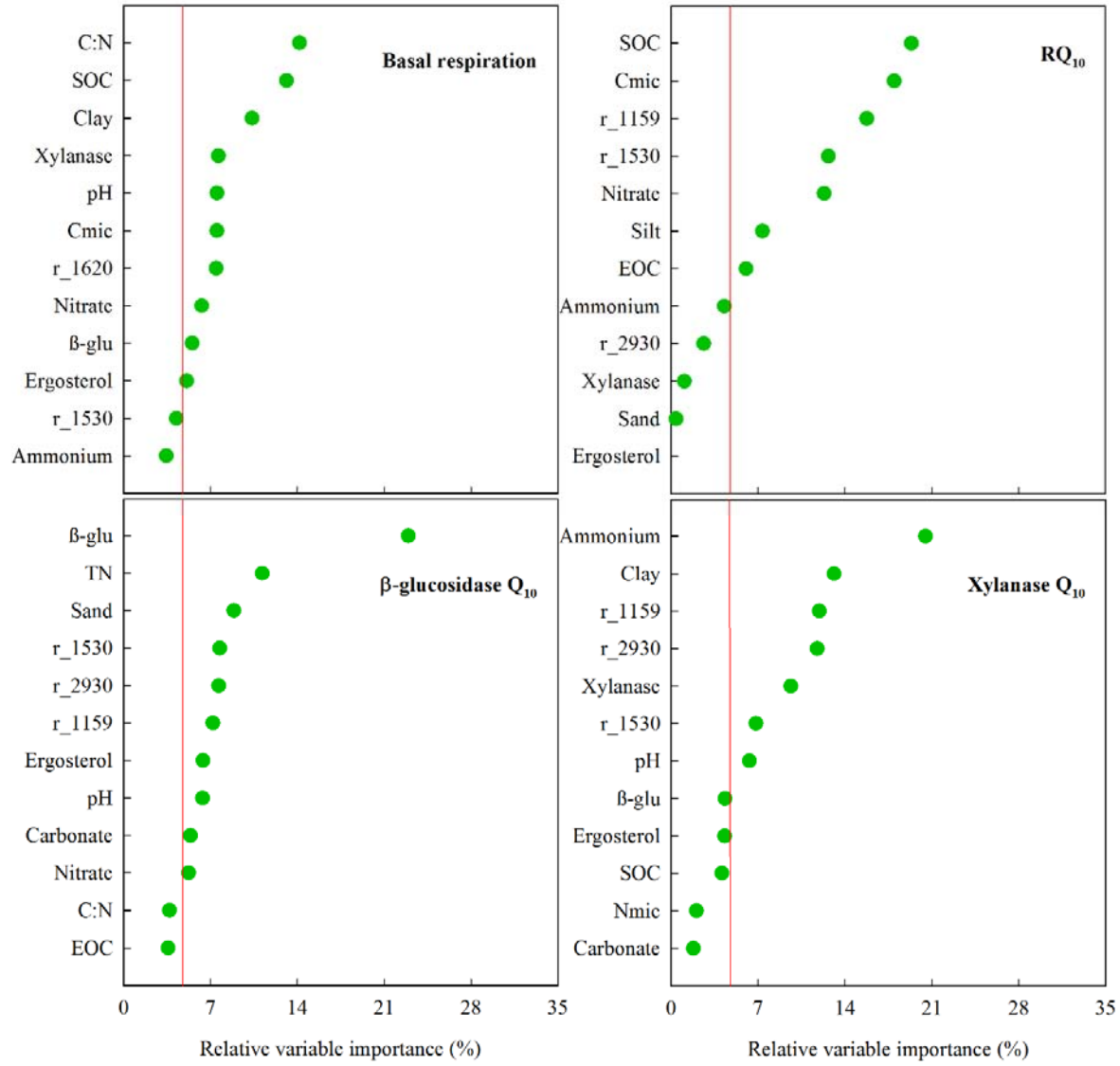

Fig. S7.6 Variable importance as given by the tree ensemble regression models using data for the regional scale. Soil respiration and activities data of both enzymes are normalized to SOC content. Lines represent theoretical importance when all explanatory variables have similar importance.

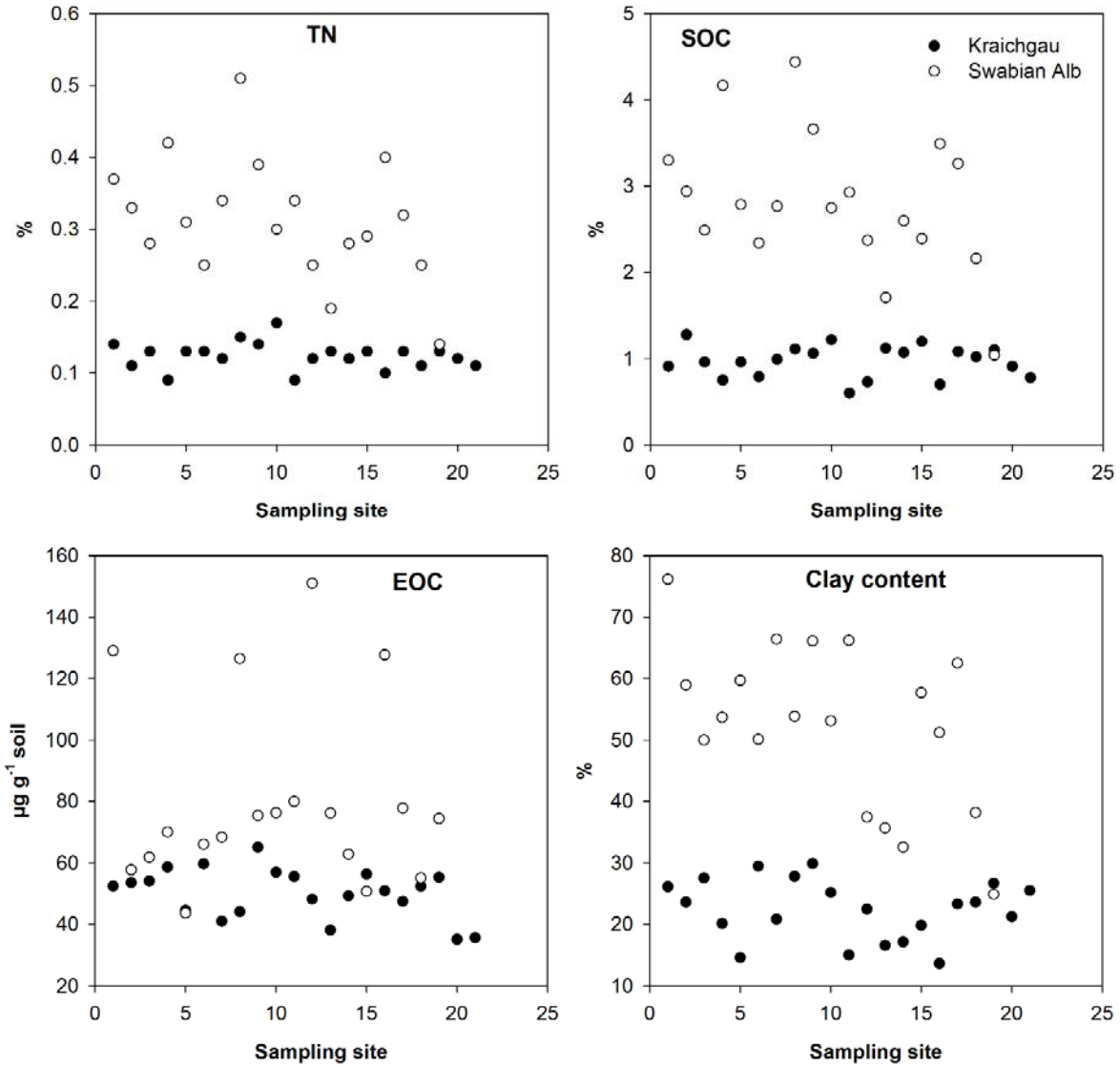

Fig. S7.7 Variation of the explaining variables between and within each landscape. Here we present examples of some variables, complete data for all explaining variables can be found in Table S1.

Table S7.1 Values of the explanatory variables from each site in both study areas. Enzyme activities, microbial and fungal biomass data were normalized on basis of soil organic carbon in each site. "x" and "y" are the Gauß-Krüger site coordinates (meters). RQ_{10}, $BetaQ_{10}$ and $XylanQ_{10}$ are the Q_{10} of soil respiration and of β-glucosidase and xylanase activities, respectively.

Area	x	y	r_2930	r_1620	r_1530	r_1159	betagluco	Xylanase	pH	SOC	Carbonate	TN	C:N
Kraichgau	3489835	5451324	14.52	71.11	4.23	10.14	320.94	397	5.58	0.91	0.06	0.14	6.52
	3490239	5449814	38.71	53.92	1.57	5.8	192.1	381.19	7.48	1.28	2.37	0.11	11.66
	3491823	5449585	3.44	81.64	4.61	11.28	415.49	255.95	7.07	0.96	0.04	0.13	7.36
	3491080	5449079	38.11	54.7	1.28	5.9	253.65	609.6	7.5	0.75	2.59	0.09	8.34
	3489251	5449185	20.44	65.4	4.42	9.74	182.81	423.34	6.53	0.96	0	0.13	7.36
	3486509	5452807	12.82	73.58	4.01	9.59	371.51	386.43	5.22	0.79	0.08	0.13	6.08
	3490275	5452795	17.84	70.73	3.14	9.77	401.39	305.56	7.31	0.99	0.19	0.12	8.25
	3492875	5453374	16.55	69.53	3.39	10.53	412.89	289.12	6.57	1.11	0.08	0.15	7.42
	3493367	5450073	6.9	78.52	3.71	10.88	507.49	259.03	6.23	1.06	0	0.14	7.54
	3493102	5447216	22.34	65.06	3.08	9.52	405.12	295.49	6.88	1.22	0.07	0.17	7.2
	3490153	5447055	7.91	76.09	4.51	11.48	217.8	772.35	5.04	0.6	0.06	0.09	6.62
	3487036	5446275	13.49	71.99	3.57	10.95	335.12	452.1	7.15	0.73	0.17	0.12	6.07
	3488459	5449890	20.68	65.8	3.3	10.23	187.37	443.87	5.37	1.12	0	0.13	8.59
	3481556	5458806	41.16	50.87	1.71	6.27	233.75	466.15	7.36	1.07	0.91	0.12	8.96
	3491253	5458990	30.04	59.14	2.39	8.42	453.63	418.28	7.33	1.2	0.4	0.13	9.25
	3499360	5458846	19.28	65.6	3.92	11.2	193.86	457.72	5.87	0.7	0	0.1	6.97
	3500061	5449895	23.13	64.99	2.56	9.31	306.52	444.07	7.37	1.08	0.25	0.13	8.31
	3499496	5440975	32.28	60.06	0.84	6.81	252.12	355.21	7.51	1.02	1.79	0.11	9.29
	3490479	5441095	17.32	71.66	1.66	9.36	436.24	766.84	7.46	1.1	0.8	0.13	8.42
	3480494	5441002	16.91	69.65	3.13	10.32	338.72	375.13	7.1	0.91	0.03	0.12	7.61
	3482390	5450274	7.42	78.71	3.07	10.81	368.58	325.41	6.26	0.78	0.06	0.11	7.12

Table S7.1 Continued

Area	C_{mic}	N_{mic}	EOC	EN	NO_3^-	NH_4^+	Clay	Sand	Silt	Ergosterol	basal respiration	RQ_{10}	$BetaQ_{10}$	$XylanQ_{10}$
Kraichgau	103.54	14.21	52.55	14.8	8.94	4.1	26.17	3.88	68.7	1.43	2.21	2.05	1.96	1.59
	162.26	21.33	53.67	15.63	11.53	1.29	23.62	2.33	75.1	0.96	1.71	2.67	1.74	2.16
	224.72	12.56	54.21	33.97	38.05	1.17	27.6	2.15	71.5	1.29	1.7	2.21	1.82	1.87
	244.51	29.45	58.7	17.97	20.97	1.16	20.14	2.78	78.1	1.47	2	2.25	1.55	2.03
	131.81	12.36	44.57	15.65	13.51	1.39	14.61	3.96	80.6	0.67	2.39	2.07	1.76	1.7
	152.05	12.38	59.78	16.91	13.77	2.16	29.51	10.91	60.3	2.72	1.97	2.02	1.84	1.53
	143.51	19.04	41.04	12.43	10.61	1.13	20.83	4.57	73.8	1.24	1.71	1.89	1.84	1.69
	196.12	14.77	44.13	32.62	39.16	1.56	27.85	2.02	69.3	1.88	1.7	1.77	1.75	1.57
	248.52	22.48	65.14	32.49	36.18	1.46	29.93	1.96	68.4	1.97	2.36	1.95	1.76	1.34
	215.72	31.48	57.06	16.65	19.33	1.13	25.22	1.94	74	1.32	1.83	1.87	2.04	1.52
	110.64	11.95	55.64	15.06	7.89	2.67	15.05	4.22	80.2	1.51	2.53	1.71	1.52	1.84
	188.58	27.77	48.24	14.68	12.86	1.3	22.48	3.43	74.4	1.17	2.26	1.73	1.85	1.89
	93.15	7.14	38.15	20.49	22.65	1.23	16.6	5.65	77.2	1	1.47	1.64	2.17	1.96
	190.98	23.05	49.32	23.9	29.79	1.23	17.17	12.8	68.6	0.9	1.72	1.64	1.68	2.8
	238.1	20.39	56.41	36.16	49.26	1.46	19.85	3.28	78.2	0.99	1.83	2.01	2.18	1.7
	165.15	20.54	50.99	19.14	20.5	2.82	13.67	3.39	82.1	0.86	2.86	1.37	1.83	1.49
	257.94	36.46	47.48	17.32	18.39	1.49	23.33	2.52	75.1	1.43	2.05	1.95	1.42	1.57
	171.24	22.67	52.52	24.03	29.73	1.35	23.62	7.25	68.2	1.15	1.69	1.75	1.53	1.5
	210.22	26.27	55.39	26	25.84	1.35	26.74	4.15	70	1.77	2.16	2.16	1.87	1.48
	167.53	24.89	35.14	13.13	13.79	1.56	21.25	4.53	74	1.69	2.12	1.71	1.99	1.65
	165.99	20.94	35.72	13.27	11.58	2.08	25.55	3.9	70	1.91	2.27	2.07	1.72	1.38

Table S7.1 Continued

Area	x	y	r_2930	r_1620	r_1530	r_1159	betagluco	Xylanase	pH	SOC	Carbonate	TN	C:N
Alb	3533589	5363681	64.74	32.14	0.25	2.97	255.48	138.56	7.36	3.3	2.72	0.37	8.91
	3534111	5364554	24.33	64.06	0.81	10.8	374.45	127.93	7.4	2.94	0.46	0.33	8.9
	3533163	5365757	34.36	53.74	2.01	9.89	188.15	157.24	6.34	2.49	0.04	0.28	8.88
	3531777	5365542	55.41	35.55	0.73	8.32	268.18	136.06	7.28	4.17	0.23	0.42	9.93
	3529367	5368142	31.99	54.97	1.9	11.14	233.02	106.02	7.02	2.79	0.09	0.31	9.01
	3534133	5368861	25.97	61.46	2.03	10.53	295.64	142.88	6.41	2.34	0.06	0.25	9.35
	3536174	5367268	58.71	37.93	-1.11	4.47	443.42	271.36	7.38	2.77	2.23	0.34	8.14
	3535962	5365210	54.57	33.24	1.49	10.7	291.98	166.26	7.16	4.44	0.22	0.51	8.7
	3532209	5359662	60.5	29.57	1.22	8.72	268.33	179.23	7.27	3.66	0.49	0.39	9.39
	3530130	5362033	33.85	54.94	1.47	9.74	372.82	214.61	7.35	2.75	0.58	0.3	9.18
	3528278	5365394	66.91	30.24	-0.13	2.98	290.57	275.97	7.46	2.93	4.23	0.34	8.62
	3524155	5374065	38.23	49.51	2.01	10.25	102.49	350.72	5.01	2.37	0.08	0.25	9.46
	3533912	5374718	24.25	61.72	2.67	11.37	258.64	337.5	5.13	1.71	0	0.19	9.02
	3542416	5374751	32.18	54.89	1.9	11.02	195.88	224.79	6.65	2.6	0.05	0.28	9.29
	3540992	5363208	35.4	51.5	1.33	11.77	337.55	164.43	7.06	2.39	0.08	0.29	8.23
	3539411	5357583	52.24	37.96	1.19	8.6	368.83	252.5	7.22	3.49	0.56	0.4	8.72
	3533385	5355846	56.64	38.69	0.25	4.55	372.24	208.8	7.18	3.26	3.41	0.32	10.2
	3524171	5356074	30.9	55.93	2.35	10.83	267.69	92.45	6.68	2.16	0.07	0.25	8.65
	3524521	5364198	16.52	67.47	3.55	12.47	151.82	236.08	6.52	1.04	0.14	0.14	7.46

Table S7.1 Continued

Area	C_{mic}	N_{mic}	EOC	EN	NO_3^-	NH_4^+	Clay	Sand	Silt	Ergosterol	basal respiration	RQ_{10}	$BetaQ_{10}$	$XylanQ_{10}$
Alb	184.48	23.01	129.07	50.06	55.53	3.33	76.13	4.16	23.6	0.85	1.36	1.77	1.84	1.59
	183.67	22.46	57.78	38.11	56.08	1.37	58.93	2.15	40	0.9	1.86	2	1.77	1.51
	154.98	16.79	61.9	29.27	44.12	1.96	49.99	2.31	48.8	1.12	1.1	1.75	1.61	1.62
	154.11	21.06	70.1	38.29	49.98	1.6	53.67	3.24	45	1.07	0.97	1.47	1.84	1.63
	132.04	8.65	43.65	49.71	52.75	1.44	59.66	2.93	38.9	0.82	0.97	2.05	1.87	1.54
	154.86	16.77	66.09	37.45	52.78	1.67	50.13	2.26	48.3	0.87	0.99	1.66	1.69	1.42
	277.65	39.82	68.42	31.49	40.19	1.47	66.44	3.21	36	1.36	2.42	1.84	1.72	1.56
	190.84	26.4	126.55	57.93	69.5	1.62	53.85	2.27	42.7	0.84	1.33	1.84	1.7	1.54
	212.65	22.99	75.41	55.64	75.12	1.51	66.12	4.48	30.7	0.87	1.62	1.99	1.84	1.77
	206.01	29.64	76.28	25.27	34.87	1.19	53.12	4.38	44.7	1.04	1.54	1.86	1.94	1.81
	211.92	25.1	79.95	61.61	80.3	2.24	66.22	8.59	34.5	0.91	1.5	1.88	1.76	1.87
	112.44	15.8	150.98	36.84	27.69	7.7	37.45	3.95	58.4	0.89	1.32	1.75	1.51	1.72
	115.23	15.41	76.22	32.11	31.32	8.95	35.69	2.43	64.2	1.02	0.74	2.07	1.66	1.83
	110.51	15.07	62.89	24.94	28.59	1.34	32.6	1.8	64.5	0.57	1.04	1.6	1.74	1.54
	206.67	25.4	50.82	31.79	41.18	1.13	57.69	2.52	37.8	1.61	1.3	2.6	1.69	1.86
	209.28	30.91	127.77	43.47	51.99	1.39	51.23	3.33	46.6	1.13	1	1.99	1.84	1.46
	194.2	28.69	77.83	32.71	45.26	1.34	62.54	8.91	36.4	0.89	1.27	2.12	1.87	1.58
	161.98	23.21	55.15	22.41	32.12	1.11	38.18	5.63	57.2	0.7	1.05	2.07	1.77	1.31
	149.13	21.94	74.43	20.68	21.92	1.78	24.91	1.74	74.1	0.46	1.65	1.91	1.59	1.69

Table S7.2 Correlations of explaining variables to basal soil respiration, its temperature sensitivity (RQ_{10}) and the temperature sensitivities of two soil enzymes from landscape and regional scale. Bold and red marked correlations are significant ($P < 0.05$).

Basal respiration in Kraichgau

	r_2930	r_1620	r_1530	r_1159	β-glucosidase	Xylanase	pH	Carbonate	SOC	TN	CN
	cm^{-1}	cm^{-1}	cm^{-1}	cm^{-1}	nmol mg^{-1} SOC h^{-1}	µg GE mg^{-1} SOC $24h^{-1}$		%	%	%	
r_2930											
r_1620	**-0.98**										
r_1530	-0.73	0.65									
r_1159	**-0.84**	**0.80**	**0.79**								
β-glucosidase	-0.44	0.43	0.01	0.15							
Xylanase	0.34	-0.28	-0.15	-0.18	-0.50						
pH	0.64	-0.59	**-0.82**	-0.70	0.06	0.08					
Carbonate	0.59	-0.54	**-0.79**	**-0.76**	0.05	0.23	**0.81**				
SOC	0.47	-0.45	-0.45	-0.52	0.23	-0.31	0.37	0.28			
TN	-0.21	0.16	0.27	0.04	0.52	-0.46	-0.24	-0.29	0.53		
CN	0.71	-0.65	**-0.76**	-0.69	-0.07	0.03	0.71	0.54	0.67	-0.12	
C_{mic}	0.12	-0.16	-0.39	-0.22	0.57	-0.15	0.53	0.36	0.29	0.22	0.28
N_{mic}	0.38	-0.35	-0.66	-0.41	0.20	0.11	0.68	0.51	0.15	-0.08	0.24
EOC	-0.03	0.01	0.01	-0.19	0.33	0.07	0.01	0.21	0.03	0.13	-0.04
EN	0.13	-0.16	-0.13	-0.14	0.33	-0.07	0.17	0.11	0.43	0.33	0.41
NO_3^-	0.17	-0.21	-0.24	-0.14	0.42	-0.20	0.27	0.10	0.45	0.34	0.46
NH_4^+	-0.41	0.31	0.38	0.38	-0.04	0.15	-0.53	-0.35	-0.42	-0.01	-0.47
Clay	-0.48	0.46	0.03	0.05	0.68	-0.61	-0.01	0.07	0.22	0.53	-0.14
Sand	0.11	-0.02	-0.07	-0.13	-0.37	0.38	-0.08	0.06	-0.27	-0.36	0.08
Silt	0.27	-0.28	0.10	0.09	-0.46	0.48	-0.03	-0.17	-0.13	-0.35	0.01
Ergosterol	-0.62	0.61	0.11	0.26	0.58	-0.25	-0.26	-0.06	-0.19	0.23	-0.34
Respiration	-0.37	0.33	0.37	0.41	-0.10	0.38	-0.39	-0.40	-0.61	-0.19	-0.59

Table S7.2 Continued

Basal respiration in Kraichgau

	C_{mic}	N_{mic}	EOC	EN	NO_3^-	NH_4^+	Clay	Sand	Silt	Ergosterol
	µg C mg^{-1} SOC	µg N mg^{-1} SOC	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	%	%	%	µg mg^{-1} SOC
r_2930										
r_1620										
r_1530										
r_1159										
β-glucosidase										
Xylanase										
pH										
Carbonate										
SOC										
TN										
CN										
C_{mic}										
N_{mic}	0.68									
EOC	0.37	0.10								
EN	0.54	-0.04	0.41							
NO_3^-	0.65	0.14	0.21	**0.92**						
NH_4^+	-0.25	-0.29	-0.04	-0.10	-0.22					
Clay	0.34	0.13	0.31	0.21	0.19	0.08				
Sand	-0.58	-0.28	-0.32	-0.27	-0.24	0.08	-0.34			
Silt	-0.11	-0.08	-0.13	-0.14	-0.19	-0.02	**-0.77**	-0.18		
Ergosterol	0.21	0.08	0.22	-0.06	-0.04	0.33	0.70	-0.11	-0.50	
Respiration	-0.08	0.07	0.11	-0.35	-0.42	0.57	-0.18	-0.09	0.31	0.17

Table S7.2 Continued

Basal respiration in Swabian Alb	r_2930	r_1620	r_1530	r_1159	β-glucosidase	Xylanase	pH	Carbonate	SOC	TN	CN
	cm^{-1}	cm^{-1}	cm^{-1}	cm^{-1}	nmol mg^{-1} SOC h^{-1}	µg GE mg^{-1} SOC $24h^{-1}$		%	%	%	
r_2930											
r_1620	**-0.97**										
r_1530	**-0.80**	0.70									
r_1159	**-0.84**	0.75	**0.78**								
β-glucosidase	0.22	-0.14	-0.58	-0.34							
Xylanase	0.17	-0.16	-0.02	-0.12	-0.04						
pH	0.58	-0.50	**-0.88**	-0.62	0.65	-0.12					
Carbonate	0.70	-0.59	**-0.82**	-0.75	0.52	0.13	**0.85**				
SOC	0.69	-0.74	-0.73	-0.61	0.37	-0.20	0.66	0.63			
TN	0.74	**-0.78**	**-0.78**	-0.63	0.45	-0.16	0.74	0.66	**0.97**		
CN	0.09	-0.10	0.00	-0.22	-0.10	-0.06	-0.17	-0.04	0.23	0.05	
C_{mic}	0.63	-0.58	-0.68	-0.56	0.72	0.09	0.71	0.70	0.47	0.59	-0.34
N_{mic}	0.50	-0.42	-0.58	-0.49	0.74	0.15	0.62	0.69	0.38	0.50	-0.38
EOC	0.50	-0.51	-0.25	-0.54	-0.02	0.58	0.14	0.49	0.34	0.31	0.21
EN	0.58	-0.64	-0.48	-0.46	0.15	-0.08	0.44	0.45	0.73	0.72	0.18
NO_3^-	0.50	-0.53	-0.55	-0.46	0.38	-0.35	0.59	0.47	0.72	0.72	0.04
NH_4^+	0.11	-0.16	0.15	-0.17	-0.51	0.38	-0.25	-0.10	-0.09	-0.09	0.06
Clay	0.73	-0.64	**-0.84**	-0.65	0.52	-0.20	**0.79**	0.75	0.65	0.70	-0.05
Sand	0.64	-0.55	-0.45	-0.64	0.21	0.07	0.39	0.59	0.31	0.31	0.23
Silt	-0.74	0.66	**0.81**	0.62	-0.51	0.19	-0.76	-0.74	-0.64	-0.69	0.03
Ergosterol	0.28	-0.22	-0.42	-0.33	0.50	0.24	0.26	0.16	0.12	0.24	-0.09
Respiration	0.28	-0.21	-0.39	-0.25	0.32	0.19	0.53	0.56	0.14	0.20	-0.39

Table S7.2 Continued

Basal respiration in Swabian Alb

	C_{mic}	N_{mic}	EOC	EN	NO_3^-	NH_4^+	Clay	Sand	Silt	Ergosterol
	µg C mg^{-1} SOC	µg N mg^{-1} SOC	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	%	%	%	µg mg^{-1} SOC
r_2930										
r_1620										
r_1530										
r_1159										
β-glucosidase										
Xylanase										
pH										
Carbonate										
SOC										
TN										
CN										
C_{mic}										
N_{mic}	**0.86**									
EOC	0.18	0.31								
EN	0.32	0.07	0.40							
NO_3^-	0.51	0.21	0.12	**0.87**						
NH_4^+	-0.29	-0.40	0.49	0.34	0.08					
Clay	0.70	0.48	0.11	0.62	0.72	-0.08				
Sand	0.49	0.45	0.39	0.28	0.22	-0.14	0.49			
Silt	-0.74	-0.49	-0.13	-0.62	-0.72	0.11	**-0.99**	-0.52		
Ergosterol	0.46	0.41	0.04	0.04	0.05	-0.03	0.24	0.16	-0.24	
Respiration	0.54	0.50	0.19	-0.01	0.14	-0.04	0.39	0.08	-0.40	0.02

Table S7.2 Continued

Basal respiration from Pooled data

	r_2930	r_1620	r_1530	r_1159	β-glucosidase	Xylanase	pH	Carbonate	SOC	TN	CN
	cm^{-1}	cm^{-1}	cm^{-1}	cm^{-1}	nmol mg^{-1} SOC h^{-1}	µg GE mg^{-1} SOC $24h^{-1}$		%	%	%	
r_2930											
r_1620	**-0.99**										
r_1530	**-0.88**	**0.84**									
r_1159	**-0.62**	0.53	**0.60**								
β-glucosidase	-0.22	0.26	-0.03	-0.12							
Xylanase	-0.40	0.45	0.45	-0.11	-0.08						
pH	0.52	-0.43	**-0.69**	**-0.66**	0.25	-0.04					
Carbonate	**0.64**	**-0.57**	**-0.76**	**-0.73**	0.16	-0.07	**0.83**				
SOC	**0.78**	**-0.81**	**-0.76**	-0.30	0.03	**-0.75**	0.30	0.40			
TN	**0.62**	**-0.67**	**-0.59**	-0.15	0.12	**-0.79**	0.10	0.24	**0.92**		
CN	**0.72**	**-0.70**	**-0.68**	-0.38	-0.23	-0.42	0.36	0.42	**0.70**	0.44	
C_{mic}	0.22	-0.19	-0.35	-0.39	**0.63**	0.00	**0.59**	0.47	0.15	0.13	-0.03
N_{mic}	0.43	-0.39	-0.56	-0.43	0.36	-0.07	**0.63**	**0.59**	0.28	0.23	0.08
EOC	0.56	**-0.59**	-0.50	-0.25	-0.01	-0.41	0.07	0.35	**0.62**	**0.66**	0.45
EN	**0.59**	**-0.63**	**-0.58**	-0.15	0.09	**-0.62**	0.20	0.31	**0.80**	**0.78**	0.56
NO_3^-	**0.63**	**-0.66**	**-0.65**	-0.19	0.16	**-0.71**	0.29	0.33	**0.82**	**0.80**	0.55
NH_4^+	-0.04	-0.02	0.12	0.09	-0.24	0.04	-0.43	-0.19	0.00	0.13	-0.08
Clay	**0.58**	**-0.60**	**-0.67**	-0.16	0.21	**-0.81**	0.18	0.35	**0.83**	**0.89**	0.43
Sand	0.17	-0.10	-0.12	-0.42	-0.09	0.28	0.13	0.28	-0.12	-0.17	0.05
Silt	**-0.64**	**0.65**	**0.70**	0.22	-0.16	**0.77**	-0.20	-0.39	**-0.81**	**-0.85**	-0.47
Ergosterol	-0.46	0.51	0.24	-0.03	**0.57**	0.39	-0.02	-0.08	-0.45	-0.37	-0.51
Respiration	-0.52	0.56	0.43	-0.02	0.25	**0.67**	0.03	-0.08	**-0.70**	**-0.62**	**-0.68**

Table S7.2 Continued

Basal respiration from Pooled data

	C_{mic}	N_{mic}	EOC	EN	NO_3^-	NH_4^+	Clay	Sand	Silt	Ergosterol
	µg C mg^{-1} SOC	µg N mg^{-1} SOC	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	%	%	%	µg mg^{-1} SOC
r_2930										
r_1620										
r_1530										
r_1159										
β-glucosidase										
Xylanase										
pH										
Carbonate										
SOC										
TN										
CN										
C_{mic}										
N_{mic}	**0.72**									
EOC	0.15	0.28								
EN	0.27	0.08	**0.64**							
NO_3^-	0.35	0.20	0.55	**0.95**						
NH_4^+	-0.31	-0.32	0.32	0.20	0.07					
Clay	0.20	0.26	**0.63**	**0.75**	**0.77**	0.15				
Sand	-0.13	0.04	-0.11	-0.17	-0.14	-0.03	-0.10			
Silt	-0.15	-0.25	**-0.58**	**-0.72**	**-0.76**	-0.12	**-0.96**	-0.09		
Ergosterol	0.32	0.12	-0.32	-0.38	-0.37	-0.03	-0.20	0.02	0.22	
Respiration	0.24	0.08	-0.43	**-0.60**	**-0.59**	0.04	**-0.56**	0.09	**0.58**	0.47

Table S7.2 Continued

RQ_{10} in Kraichgau

	r_2930	r_1620	r_1530	r_1159	β-glucosidase	Xylanase	pH	Carbonate	SOC	TN	CN
	cm^{-1}	cm^{-1}	cm^{-1}	cm^{-1}	nmol mg^{-1} SOC h^{-1}	µg GE mg^{-1} SOC $24h^{-1}$		%	%	%	
r_2930											
r_1620	**-0.98**										
r_1530	-0.73	0.65									
r_1159	**-0.84**	**0.80**	**0.79**								
β-glucosidase	-0.44	0.43	0.01	0.15							
Xylanase	0.34	-0.28	-0.15	-0.18	-0.50						
pH	0.64	-0.59	**-0.82**	-0.70	0.06	0.08					
Carbonate	0.59	-0.54	**-0.79**	**-0.76**	0.05	0.23	**0.81**				
SOC	0.47	-0.45	-0.45	-0.52	0.23	-0.31	0.37	0.28			
TN	-0.21	0.16	0.27	0.04	0.52	-0.46	-0.24	-0.29	0.53		
CN	0.71	-0.65	**-0.76**	-0.69	-0.07	0.03	0.71	0.54	0.67	-0.12	
C_{mic}	0.12	-0.16	-0.39	-0.22	0.57	-0.15	0.53	0.36	0.29	0.22	0.28
N_{mic}	0.38	-0.35	-0.66	-0.41	0.20	0.11	0.68	0.51	0.15	-0.08	0.24
EOC	-0.03	0.01	0.01	-0.19	0.33	0.07	0.01	0.21	0.03	0.13	-0.04
EN	0.13	-0.16	-0.13	-0.14	0.33	-0.07	0.17	0.11	0.43	0.33	0.41
NO_3^-	0.17	-0.21	-0.24	-0.14	0.42	-0.20	0.27	0.10	0.45	0.34	0.46
NH_4^+	-0.41	0.31	0.38	0.38	-0.04	0.15	-0.53	-0.35	-0.42	-0.01	-0.47
Clay	-0.48	0.46	0.03	0.05	0.68	-0.61	-0.01	0.07	0.22	0.53	-0.14
Sand	0.11	-0.02	-0.07	-0.13	-0.37	0.38	-0.08	0.06	-0.27	-0.36	0.08
Silt	0.27	-0.28	0.10	0.09	-0.46	0.48	-0.03	-0.17	-0.13	-0.35	0.01
Ergosterol	-0.62	0.61	0.11	0.26	0.58	-0.25	-0.26	-0.06	-0.19	0.23	-0.34
RQ_{10}	-0.06	0.08	-0.13	-0.33	0.25	-0.18	0.29	0.30	0.13	0.11	0.11

Table S7.2 Continued

RQ_{10} in Kraichgau

	C_{mic}	N_{mic}	EOC	EN	NO_3^-	NH_4^+	Clay	Sand	Silt	Ergosterol
	µg C mg^{-1} SOC	µg N mg^{-1} SOC	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	%	%	%	µg mg^{-1} SOC
r_2930										
r_1620										
r_1530										
r_1159										
β-glucosidase										
Xylanase										
pH										
Carbonate										
SOC										
TN										
CN										
C_{mic}										
N_{mic}	0.68									
EOC	0.37	0.10								
EN	0.54	-0.04	0.41							
NO_3^-	0.65	0.14	0.21	**0.92**						
NH_4^+	-0.25	-0.29	-0.04	-0.10	-0.22					
Clay	0.34	0.13	0.31	0.21	0.19	0.08				
Sand	-0.58	-0.28	-0.32	-0.27	-0.24	0.08	-0.34			
Silt	-0.11	-0.08	-0.13	-0.14	-0.19	-0.02	**-0.77**	-0.18		
Ergosterol	0.21	0.08	0.22	-0.06	-0.04	0.33	0.70	-0.11	-0.50	
RQ_{10}	0.22	0.04	0.35	0.05	-0.08	-0.15	0.44	-0.35	-0.09	0.21

Table S7.2 Continued

RQ_{10} in Swabian Alb

	r_2930	r_1620	r_1530	r_1159	β-glucosidase	Xylanase	pH	Carbonate	SOC	TN	CN
	cm^{-1}	cm^{-1}	cm^{-1}	cm^{-1}	nmol mg^{-1} SOC h^{-1}	µg GE mg^{-1} SOC $24h^{-1}$		%	%	%	
r_2930											
r_1620	**-0.97**										
r_1530	**-0.80**	0.70									
r_1159	**-0.84**	0.75	**0.78**								
β-glucosidase	0.22	-0.14	-0.58	-0.34							
Xylanase	0.17	-0.16	-0.02	-0.12	-0.04						
pH	0.58	-0.50	**-0.88**	-0.62	0.65	-0.12					
Carbonate	0.70	-0.59	**-0.82**	-0.75	0.52	0.13	**0.85**				
SOC	0.69	-0.74	-0.73	-0.61	0.37	-0.20	0.66	0.63			
TN	0.74	**-0.78**	**-0.78**	-0.63	0.45	-0.16	0.74	0.66	**0.97**		
CN	0.09	-0.10	0.00	-0.22	-0.10	-0.06	-0.17	-0.04	0.23	0.05	
C_{mic}	0.63	-0.58	-0.68	-0.56	0.72	0.09	0.71	0.70	0.47	0.59	-0.34
N_{mic}	0.50	-0.42	-0.58	-0.49	0.74	0.15	0.62	0.69	0.38	0.50	-0.38
EOC	0.50	-0.51	-0.25	-0.54	-0.02	0.58	0.14	0.49	0.34	0.31	0.21
EN	0.58	-0.64	-0.48	-0.46	0.15	-0.08	0.44	0.45	0.73	0.72	0.18
NO_3^-	0.50	-0.53	-0.55	-0.46	0.38	-0.35	0.59	0.47	0.72	0.72	0.04
NH_4^+	0.11	-0.16	0.15	-0.17	-0.51	0.38	-0.25	-0.10	-0.09	-0.09	0.06
Clay	0.73	-0.64	**-0.84**	-0.65	0.52	-0.20	**0.79**	0.75	0.65	0.70	-0.05
Sand	0.64	-0.55	-0.45	-0.64	0.21	0.07	0.39	0.59	0.31	0.31	0.23
Silt	-0.74	0.66	**0.81**	0.62	-0.51	0.19	**-0.76**	-0.74	-0.64	-0.69	0.03
Ergosterol	0.28	-0.22	-0.42	-0.33	0.50	0.24	0.26	0.16	0.12	0.24	-0.09
RQ_{10}	-0.18	0.23	0.04	0.32	0.30	-0.07	0.06	0.09	-0.13	-0.12	-0.26

Table S7.2 Continued

RQ_{10} in Swabian Alb

	C_{mic} µg C mg^{-1} SOC	N_{mic} µg N mg^{-1} SOC	EOC µg g^{-1} soil	EN µg g^{-1} soil	NO_3^- µg g^{-1} soil	NH_4^+ µg g^{-1} soil	Clay %	Sand %	Silt %	Ergosterol µg mg^{-1} SOC
r_2930										
r_1620										
r_1530										
r_1159										
β-glucosidase										
Xylanase										
pH										
Carbonate										
SOC										
TN										
CN										
C_{mic}										
N_{mic}	**0.86**									
EOC	0.18	0.31								
EN	0.32	0.07	0.40							
NO_3^-	0.51	0.21	0.12	**0.87**						
NH_4^+	-0.29	-0.40	0.49	0.34	0.08					
Clay	0.70	0.48	0.11	0.62	0.72	-0.08				
Sand	0.49	0.45	0.39	0.28	0.22	-0.14	0.49			
Silt	-0.74	-0.49	-0.13	-0.62	-0.72	0.11	**-0.99**	-0.52		
Ergosterol	0.46	0.41	0.04	0.04	0.05	-0.03	0.24	0.16	-0.24	
RQ_{10}	0.29	0.27	-0.22	-0.05	0.01	-0.41	0.17	0.27	-0.22	0.05

Table S7.2 Continued

RQ_{10} in Pooled data

	r_2930	r_1620	r_1530	r_1159	β-glucosidase	Xylanase	pH	Carbonate	SOC	TN	CN
	cm^{-1}	cm^{-1}	cm^{-1}	cm^{-1}	nmol mg^{-1} SOC h^{-1}	µg GE mg^{-1} SOC $24h^{-1}$		%	%	%	
r_2930											
r_1620	**-0.99**										
r_1530	**-0.88**	**0.84**									
r_1159	**-0.62**	0.53	**0.60**								
β-glucosidase	-0.22	0.26	-0.03	-0.12							
Xylanase	-0.40	0.45	0.45	-0.11	-0.08						
pH	0.52	-0.43	**-0.69**	**-0.66**	0.25	-0.04					
Carbonate	**0.64**	**-0.57**	**-0.76**	**-0.73**	0.16	-0.07	**0.83**				
SOC	**0.78**	**-0.81**	**-0.76**	-0.30	0.03	**-0.75**	0.30	0.40			
TN	**0.62**	**-0.67**	**-0.59**	-0.15	0.12	**-0.79**	0.10	0.24	**0.92**		
CN	**0.72**	**-0.70**	**-0.68**	-0.38	-0.23	-0.42	0.36	0.42	**0.70**	0.44	
C_{mic}	0.22	-0.19	-0.35	-0.39	**0.63**	0.00	**0.59**	0.47	0.15	0.13	-0.03
N_{mic}	0.43	-0.39	-0.56	-0.43	0.36	-0.07	**0.63**	**0.59**	0.28	0.23	0.08
EOC	0.56	**-0.59**	-0.50	-0.25	-0.01	-0.41	0.07	0.35	**0.62**	**0.66**	0.45
EN	**0.59**	**-0.63**	**-0.58**	-0.15	0.09	**-0.62**	0.20	0.31	**0.80**	**0.78**	0.56
NO_3^-	**0.63**	**-0.66**	**-0.65**	-0.19	0.16	**-0.71**	0.29	0.33	**0.82**	**0.80**	0.55
NH_4^+	-0.04	-0.02	0.12	0.09	-0.24	0.04	-0.43	-0.19	0.00	0.13	-0.08
Clay	**0.58**	**-0.60**	**-0.67**	-0.16	0.21	**-0.81**	0.18	0.35	**0.83**	**0.89**	0.43
Sand	0.17	-0.10	-0.12	-0.42	-0.09	0.28	0.13	0.28	-0.12	-0.17	0.05
Silt	**-0.64**	**0.65**	**0.70**	0.22	-0.16	**0.77**	-0.20	-0.39	**-0.81**	**-0.85**	-0.47
Ergosterol	-0.46	0.51	0.24	-0.03	**0.57**	0.39	-0.02	-0.08	-0.45	-0.37	-0.51
RQ_{10}	-0.07	0.10	-0.03	-0.03	0.28	-0.04	0.17	0.17	-0.03	-0.03	-0.05

Table S7.2 Continued

RQ_{10} in Pooled data

	C_{mic} µg C mg^{-1} SOC	N_{mic} µg N mg^{-1} SOC	EOC µg g^{-1} soil	EN µg g^{-1} soil	NO_3^- µg g^{-1} soil	NH_4^+ µg g^{-1} soil	Clay %	Sand %	Silt %	Ergosterol µg mg^{-1} SOC
r_2930										
r_1620										
r_1530										
r_1159										
β-glucosidase										
Xylanase										
pH										
Carbonate										
SOC										
TN										
CN										
C_{mic}										
N_{mic}	**0.72**									
EOC	0.15	0.28								
EN	0.27	0.08	**0.64**							
NO_3^-	0.35	0.20	0.55	**0.95**						
NH_4^+	-0.31	-0.32	0.32	0.20	0.07					
Clay	0.20	0.26	**0.63**	**0.75**	**0.77**	0.15				
Sand	-0.13	0.04	-0.11	-0.17	-0.14	-0.03	-0.10			
Silt	-0.15	-0.25	**-0.58**	**-0.72**	**-0.76**	-0.12	**-0.96**	-0.09		
Ergosterol	0.32	0.12	-0.32	-0.38	-0.37	-0.03	-0.20	0.02	0.22	
RQ_{10}	0.26	0.10	-0.04	-0.02	-0.04	-0.26	0.13	-0.07	-0.03	0.17

Table S7.2 Continued

β-glucosidase Q_{10} in Kraichgau

	r_2930	r_1620	r_1530	r_1159	β-glucosidase	Xylanase	pH	Carbonate	SOC	TN	CN
	cm^{-1}	cm^{-1}	cm^{-1}	cm^{-1}	nmol mg^{-1} SOC h^{-1}	µg GE mg^{-1} SOC $24h^{-1}$		%	%	%	
r_2930											
r_1620	**-0.98**										
r_1530	-0.73	0.65									
r_1159	**-0.84**	**0.80**	**0.79**								
β-glucosidase	-0.44	0.43	0.01	0.15							
Xylanase	0.34	-0.28	-0.15	-0.18	-0.50						
pH	0.64	-0.59	**-0.82**	-0.70	0.06	0.08					
Carbonate	0.59	-0.54	**-0.79**	**-0.76**	0.05	0.23	**0.81**				
SOC	0.47	-0.45	-0.45	-0.52	0.23	-0.31	0.37	0.28			
TN	-0.21	0.16	0.27	0.04	0.52	-0.46	-0.24	-0.29	0.53		
CN	0.71	-0.65	**-0.76**	-0.69	-0.07	0.03	0.71	0.54	0.67	-0.12	
C_{mic}	0.12	-0.16	-0.39	-0.22	0.57	-0.15	0.53	0.36	0.29	0.22	0.28
N_{mic}	0.38	-0.35	-0.66	-0.41	0.20	0.11	0.68	0.51	0.15	-0.08	0.24
EOC	-0.03	0.01	0.01	-0.19	0.33	0.07	0.01	0.21	0.03	0.13	-0.04
EN	0.13	-0.16	-0.13	-0.14	0.33	-0.07	0.17	0.11	0.43	0.33	0.41
NO_3^-	0.17	-0.21	-0.24	-0.14	0.42	-0.20	0.27	0.10	0.45	0.34	0.46
NH_4^+	-0.41	0.31	0.38	0.38	-0.04	0.15	-0.53	-0.35	-0.42	-0.01	-0.47
Clay	-0.48	0.46	0.03	0.05	0.68	-0.61	-0.01	0.07	0.22	0.53	-0.14
Sand	0.11	-0.02	-0.07	-0.13	-0.37	0.38	-0.08	0.06	-0.27	-0.36	0.08
Silt	0.27	-0.28	0.10	0.09	-0.46	0.48	-0.03	-0.17	-0.13	-0.35	0.01
Ergosterol	-0.62	0.61	0.11	0.26	0.58	-0.25	-0.26	-0.06	-0.19	0.23	-0.34
β-gluco Q_{10}	-0.12	0.14	0.18	0.12	0.34	-0.15	-0.25	-0.33	0.24	0.48	-0.12

Table S7.2 Continued

β-glucosidase Q_{10} in Kraichgau

	C_{mic}	N_{mic}	EOC	EN	NO_3^-	NH_4^+	Clay	Sand	Silt	Ergosterol
	µg C mg^{-1} SOC	µg N mg^{-1} SOC	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	%	%	%	µg mg^{-1} SOC
r_2930										
r_1620										
r_1530										
r_1159										
β-glucosidase										
Xylanase										
pH										
Carbonate										
SOC										
TN										
CN										
C_{mic}										
N_{mic}	0.68									
EOC	0.37	0.10								
EN	0.54	-0.04	0.41							
NO_3^-	0.65	0.14	0.21	**0.92**						
NH_4^+	-0.25	-0.29	-0.04	-0.10	-0.22					
Clay	0.34	0.13	0.31	0.21	0.19	0.08				
Sand	-0.58	-0.28	-0.32	-0.27	-0.24	0.08	-0.34			
Silt	-0.11	-0.08	-0.13	-0.14	-0.19	-0.02	**-0.77**	-0.18		
Ergosterol	0.21	0.08	0.22	-0.06	-0.04	0.33	0.70	-0.11	-0.50	
β-gluco Q_{10}	-0.17	-0.14	-0.01	-0.01	0.09	-0.08	0.04	0.01	0.06	-0.06

Table S7.2 Continued

β-glucosidase Q_{10} in Swabian Alb

	r_2930	r_1620	r_1530	r_1159	β-glucosidase	Xylanase	pH	Carbonate	SOC	TN	CN
	cm^{-1}	cm^{-1}	cm^{-1}	cm^{-1}	nmol mg^{-1} SOC h^{-1}	µg GE mg^{-1} SOC $24h^{-1}$		%	%	%	
r_2930											
r_1620	**-0.97**										
r_1530	**-0.80**	0.70									
r_1159	**-0.84**	0.75	**0.78**								
β-glucosidase	0.22	-0.14	-0.58	-0.34							
Xylanase	0.17	-0.16	-0.02	-0.12	-0.04						
pH	0.58	-0.50	**-0.88**	-0.62	0.65	-0.12					
Carbonate	0.70	-0.59	**-0.82**	-0.75	0.52	0.13	**0.85**				
SOC	0.69	-0.74	-0.73	-0.61	0.37	-0.20	0.66	0.63			
TN	0.74	**-0.78**	**-0.78**	-0.63	0.45	-0.16	0.74	0.66	**0.97**		
CN	0.09	-0.10	0.00	-0.22	-0.10	-0.06	-0.17	-0.04	0.23	0.05	
C_{mic}	0.63	-0.58	-0.68	-0.56	0.72	0.09	0.71	0.70	0.47	0.59	-0.34
N_{mic}	0.50	-0.42	-0.58	-0.49	0.74	0.15	0.62	0.69	0.38	0.50	-0.38
EOC	0.50	-0.51	-0.25	-0.54	-0.02	0.58	0.14	0.49	0.34	0.31	0.21
EN	0.58	-0.64	-0.48	-0.46	0.15	-0.08	0.44	0.45	0.73	0.72	0.18
NO_3^-	0.50	-0.53	-0.55	-0.46	0.38	-0.35	0.59	0.47	0.72	0.72	0.04
NH_4^+	0.11	-0.16	0.15	-0.17	-0.51	0.38	-0.25	-0.10	-0.09	-0.09	0.06
Clay	0.73	-0.64	**-0.84**	-0.65	0.52	-0.20	**0.79**	0.75	0.65	0.70	-0.05
Sand	0.64	-0.55	-0.45	-0.64	0.21	0.07	0.39	0.59	0.31	0.31	0.23
Silt	-0.74	0.66	**0.81**	0.62	-0.51	0.19	-0.76	-0.74	-0.64	-0.69	0.03
Ergosterol	0.28	-0.22	-0.42	-0.33	0.50	0.24	0.26	0.16	0.12	0.24	-0.09
β-gluco Q_{10}	0.34	-0.28	-0.52	-0.43	0.43	-0.37	0.62	0.60	0.61	0.55	0.33

Table S7.2 Continued

β-glucosidase Q_{10} in Swabian Alb

	C_{mic}	N_{mic}	EOC	EN	NO_3^-	NH_4^+	Clay	Sand	Silt	Ergosterol
	µg C mg^{-1} SOC	µg N mg^{-1} SOC	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	%	%	%	µg mg^{-1} SOC
r_2930										
r_1620										
r_1530										
r_1159										
β-glucosidase										
Xylanase										
pH										
Carbonate										
SOC										
TN										
CN										
C_{mic}										
N_{mic}	**0.86**									
EOC	0.18	0.31								
EN	0.32	0.07	0.40							
NO_3^-	0.51	0.21	0.12	**0.87**						
NH_4^+	-0.29	-0.40	0.49	0.34	0.08					
Clay	0.70	0.48	0.11	0.62	0.72	-0.08				
Sand	0.49	0.45	0.39	0.28	0.22	-0.14	0.49			
Silt	-0.74	-0.49	-0.13	-0.62	-0.72	0.11	**-0.99**	-0.52		
Ergosterol	0.46	0.41	0.04	0.04	0.05	-0.03	0.24	0.16	-0.24	
β-gluco Q_{10}	0.36	0.33	0.03	0.31	0.41	-0.51	0.54	0.55	-0.53	-0.03

Table S7.2 Continued

β-glucosidase Q_{10} in Pooled data

	r_2930	r_1620	r_1530	r_1159	β-glucosidase	Xylanase	pH	Carbonate	SOC	TN	CN
	cm^{-1}	cm^{-1}	cm^{-1}	cm^{-1}	nmol mg^{-1} SOC h^{-1}	µg GE mg^{-1} SOC $24h^{-1}$		%	%	%	
r_2930											
r_1620	**-0.99**										
r_1530	**-0.88**	**0.84**									
r_1159	**-0.62**	0.53	**0.60**								
β-glucosidase	-0.22	0.26	-0.03	-0.12							
Xylanase	-0.40	0.45	0.45	-0.11	-0.08						
pH	0.52	-0.43	**-0.69**	**-0.66**	0.25	-0.04					
Carbonate	**0.64**	**-0.57**	**-0.76**	**-0.73**	0.16	-0.07	**0.83**				
SOC	**0.78**	**-0.81**	**-0.76**	-0.30	0.03	**-0.75**	0.30	0.40			
TN	**0.62**	**-0.67**	**-0.59**	-0.15	0.12	**-0.79**	0.10	0.24	**0.92**		
CN	**0.72**	**-0.70**	**-0.68**	-0.38	-0.23	-0.42	0.36	0.42	**0.70**	0.44	
C_{mic}	0.22	-0.19	-0.35	-0.39	**0.63**	0.00	**0.59**	0.47	0.15	0.13	-0.03
N_{mic}	0.43	-0.39	-0.56	-0.43	0.36	-0.07	**0.63**	**0.59**	0.28	0.23	0.08
EOC	0.56	**-0.59**	-0.50	-0.25	-0.01	-0.41	0.07	0.35	**0.62**	**0.66**	0.45
EN	**0.59**	**-0.63**	**-0.58**	-0.15	0.09	**-0.62**	0.20	0.31	**0.80**	**0.78**	0.56
NO_3^-	**0.63**	**-0.66**	**-0.65**	-0.19	0.16	**-0.71**	0.29	0.33	**0.82**	**0.80**	0.55
NH_4^+	-0.04	-0.02	0.12	0.09	-0.24	0.04	-0.43	-0.19	0.00	0.13	-0.08
Clay	**0.58**	**-0.60**	**-0.67**	-0.16	0.21	**-0.81**	0.18	0.35	**0.83**	**0.89**	0.43
Sand	0.17	-0.10	-0.12	-0.42	-0.09	0.28	0.13	0.28	-0.12	-0.17	0.05
Silt	**-0.64**	**0.65**	**0.70**	0.22	-0.16	**0.77**	-0.20	-0.39	**-0.81**	**-0.85**	-0.47
Ergosterol	-0.46	0.51	0.24	-0.03	**0.57**	0.39	-0.02	-0.08	-0.45	-0.37	-0.51
β-gluco Q_{10}	-0.10	0.13	0.07	-0.16	0.42	-0.06	0.05	-0.01	0.10	0.14	-0.07

Table S7.2 Continued

β-glucosidase Q_{10} in Pooled data

	C_{mic}	N_{mic}	EOC	EN	NO_3^-	NH_4^+	Clay	Sand	Silt	Ergosterol
	µg C mg^{-1} SOC	µg N mg^{-1} SOC	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	%	%	%	µg mg^{-1} SOC
r_2930										
r_1620										
r_1530										
r_1159										
β-glucosidase										
Xylanase										
pH										
Carbonate										
SOC										
TN										
CN										
C_{mic}										
N_{mic}	**0.72**									
EOC	0.15	0.28								
EN	0.27	0.08	**0.64**							
NO_3^-	0.35	0.20	0.55	**0.95**						
NH_4^+	-0.31	-0.32	0.32	0.20	0.07					
Clay	0.20	0.26	**0.63**	**0.75**	**0.77**	0.15				
Sand	-0.13	0.04	-0.11	-0.17	-0.14	-0.03	-0.10			
Silt	-0.15	-0.25	**-0.58**	**-0.72**	**-0.76**	-0.12	**-0.96**	-0.09		
Ergosterol	0.32	0.12	-0.32	-0.38	-0.37	-0.03	-0.20	0.02	0.22	
β-gluco Q_{10}	0.05	0.01	-0.13	-0.01	0.05	-0.24	0.04	0.26	-0.02	0.03

Table S7.2 Continued

Xylanase Q_{10} in Kraichgau

	r_2930	r_1620	r_1530	r_1159	β-glucosidase	Xylanase µg GE mg^{-1}	pH	Carbonate	SOC	TN	CN
	cm^{-1}	cm^{-1}	cm^{-1}	cm^{-1}	nmol mg^{-1} SOC h^{-1}	SOC $24h^{-1}$		%	%	%	
r_2930											
r_1620	**-0.98**										
r_1530	-0.73	0.65									
r_1159	**-0.84**	**0.80**	**0.79**								
β-glucosidase	-0.44	0.43	0.01	0.15							
Xylanase	0.34	-0.28	-0.15	-0.18	-0.50						
pH	0.64	-0.59	**-0.82**	-0.70	0.06	0.08					
Carbonate	0.59	-0.54	**-0.79**	**-0.76**	0.05	0.23	**0.81**				
SOC	0.47	-0.45	-0.45	-0.52	0.23	-0.31	0.37	0.28			
TN	-0.21	0.16	0.27	0.04	0.52	-0.46	-0.24	-0.29	0.53		
CN	0.71	-0.65	**-0.76**	-0.69	-0.07	0.03	0.71	0.54	0.67	-0.12	
C_{mic}	0.12	-0.16	-0.39	-0.22	0.57	-0.15	0.53	0.36	0.29	0.22	0.28
N_{mic}	0.38	-0.35	-0.66	-0.41	0.20	0.11	0.68	0.51	0.15	-0.08	0.24
EOC	-0.03	0.01	0.01	-0.19	0.33	0.07	0.01	0.21	0.03	0.13	-0.04
EN	0.13	-0.16	-0.13	-0.14	0.33	-0.07	0.17	0.11	0.43	0.33	0.41
NO_3^-	0.17	-0.21	-0.24	-0.14	0.42	-0.20	0.27	0.10	0.45	0.34	0.46
NH_4^+	-0.41	0.31	0.38	0.38	-0.04	0.15	-0.53	-0.35	-0.42	-0.01	-0.47
Clay	-0.48	0.46	0.03	0.05	0.68	-0.61	-0.01	0.07	0.22	0.53	-0.14
Sand	0.11	-0.02	-0.07	-0.13	-0.37	0.38	-0.08	0.06	-0.27	-0.36	0.08
Silt	0.27	-0.28	0.10	0.09	-0.46	0.48	-0.03	-0.17	-0.13	-0.35	0.01
Ergosterol	-0.62	0.61	0.11	0.26	0.58	-0.25	-0.26	-0.06	-0.19	0.23	-0.34
Xylanase Q_{10}	0.39	-0.40	-0.04	-0.24	-0.46	0.33	0.22	0.27	0.05	-0.28	0.26

Table S7.2 Continued

Xylanase Q_{10} in Kraichgau

	C_{mic}	N_{mic}	EOC	EN	NO_3^-	NH_4^+	Clay	Sand	Silt	Ergosterol
	µg C mg^{-1} SOC	µg N mg^{-1} SOC	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	%	%	%	µg mg^{-1} SOC
r_2930										
r_1620										
r_1530										
r_1159										
β-glucosidase										
Xylanase										
pH										
Carbonate										
SOC										
TN										
CN										
C_{mic}										
N_{mic}	0.68									
EOC	0.37	0.10								
EN	0.54	-0.04	0.41							
NO_3^-	0.65	0.14	0.21	**0.92**						
NH_4^+	-0.25	-0.29	-0.04	-0.10	-0.22					
Clay	0.34	0.13	0.31	0.21	0.19	0.08				
Sand	-0.58	-0.28	-0.32	-0.27	-0.24	0.08	-0.34			
Silt	-0.11	-0.08	-0.13	-0.14	-0.19	-0.02	**-0.77**	-0.18		
Ergosterol	0.21	0.08	0.22	-0.06	-0.04	0.33	0.70	-0.11	-0.50	
Xylanase Q_{10}	-0.18	-0.15	-0.09	-0.09	-0.09	-0.44	-0.48	0.14	0.37	-0.50

Table S7.2 Continued

Xylanase Q_{10} in Swabian Alb

	r_2930	r_1620	r_1530	r_1159	β-glucosidase	Xylanase	pH	Carbonate	SOC	TN	CN
	cm^{-1}	cm^{-1}	cm^{-1}	cm^{-1}	nmol mg^{-1} SOC h^{-1}	µg GE mg^{-1} SOC $24h^{-1}$		%	%	%	
r_2930											
r_1620	**-0.97**										
r_1530	**-0.80**	0.70									
r_1159	**-0.84**	0.75	**0.78**								
β-glucosidase	0.22	-0.14	-0.58	-0.34							
Xylanase	0.17	-0.16	-0.02	-0.12	-0.04						
pH	0.58	-0.50	**-0.88**	-0.62	0.65	-0.12					
Carbonate	0.70	-0.59	**-0.82**	-0.75	0.52	0.13	**0.85**				
SOC	0.69	-0.74	-0.73	-0.61	0.37	-0.20	0.66	0.63			
TN	0.74	**-0.78**	**-0.78**	-0.63	0.45	-0.16	0.74	0.66	**0.97**		
CN	0.09	-0.10	0.00	-0.22	-0.10	-0.06	-0.17	-0.04	0.23	0.05	
C_{mic}	0.63	-0.58	-0.68	-0.56	0.72	0.09	0.71	0.70	0.47	0.59	-0.34
N_{mic}	0.50	-0.42	-0.58	-0.49	0.74	0.15	0.62	0.69	0.38	0.50	-0.38
EOC	0.50	-0.51	-0.25	-0.54	-0.02	0.58	0.14	0.49	0.34	0.31	0.21
EN	0.58	-0.64	-0.48	-0.46	0.15	-0.08	0.44	0.45	0.73	0.72	0.18
NO_3^-	0.50	-0.53	-0.55	-0.46	0.38	-0.35	0.59	0.47	0.72	0.72	0.04
NH_4^+	0.11	-0.16	0.15	-0.17	-0.51	0.38	-0.25	-0.10	-0.09	-0.09	0.06
Clay	0.73	-0.64	**-0.84**	-0.65	0.52	-0.20	**0.79**	0.75	0.65	0.70	-0.05
Sand	0.64	-0.55	-0.45	-0.64	0.21	0.07	0.39	0.59	0.31	0.31	0.23
Silt	-0.74	0.66	**0.81**	0.62	-0.51	0.19	-0.76	-0.74	-0.64	-0.69	0.03
Ergosterol	0.28	-0.22	-0.42	-0.33	0.50	0.24	0.26	0.16	0.12	0.24	-0.09
Xylanase Q_{10}	0.27	-0.26	-0.10	-0.06	-0.15	0.49	0.06	0.14	-0.11	-0.08	0.00

Table S7.2 Continued

Xylanase Q_{10} in Swabian Alb

	C_{mic}	N_{mic}	EOC	EN	NO_3^-	NH_4^+	Clay	Sand	Silt	Erg
	µg C mg^{-1} SOC	µg N mg^{-1} SOC	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	%	%	%	µg n
r_2930										
r_1620										
r_1530										
r_1159										
β-glucosidase										
Xylanase										
pH										
Carbonate										
SOC										
TN										
CN										
C_{mic}										
N_{mic}	**0.86**									
EOC	0.18	0.31								
EN	0.32	0.07	0.40							
NO_3^-	0.51	0.21	0.12	**0.87**						
NH_4^+	-0.29	-0.40	0.49	0.34	0.08					
Clay	0.70	0.48	0.11	0.62	0.72	-0.08				
Sand	0.49	0.45	0.39	0.28	0.22	-0.14	0.49			
Silt	-0.74	-0.49	-0.13	-0.62	-0.72	0.11	**-0.99**	-0.52		
Ergosterol	0.46	0.41	0.04	0.04	0.05	-0.03	0.24	0.16	-0.24	
Xylanase Q_{10}	0.15	0.01	0.26	0.04	-0.11	0.34	0.10	0.24	-0.17	

Table S7.2 Continued

Xylanase Q_{10} in Pooled data

	r_2930	r_1620	r_1530	r_1159	β-glucosidase	Xylanase	pH	Carbonate	SOC	TN	CN
	cm^{-1}	cm^{-1}	cm^{-1}	cm^{-1}	nmol mg^{-1} SOC h^{-1}	µg GE mg^{-1} SOC $24h^{-1}$		%	%	%	
r_2930											
r_1620	**-0.99**										
r_1530	**-0.88**	**0.84**									
r_1159	**-0.62**	0.53	**0.60**								
β-glucosidase	-0.22	0.26	-0.03	-0.12							
Xylanase	-0.40	0.45	0.45	-0.11	-0.08						
pH	0.52	-0.43	**-0.69**	**-0.66**	0.25	-0.04					
Carbonate	**0.64**	**-0.57**	**-0.76**	**-0.73**	0.16	-0.07	**0.83**				
SOC	**0.78**	**-0.81**	**-0.76**	-0.30	0.03	**-0.75**	0.30	0.40			
TN	**0.62**	**-0.67**	**-0.59**	-0.15	0.12	**-0.79**	0.10	0.24	**0.92**		
CN	**0.72**	**-0.70**	**-0.68**	-0.38	-0.23	-0.42	0.36	0.42	**0.70**	0.44	
C_{mic}	0.22	-0.19	-0.35	-0.39	**0.63**	0.00	**0.59**	0.47	0.15	0.13	-0.03
N_{mic}	0.43	-0.39	-0.56	-0.43	0.36	-0.07	**0.63**	**0.59**	0.28	0.23	0.08
EOC	0.56	**-0.59**	-0.50	-0.25	-0.01	-0.41	0.07	0.35	**0.62**	**0.66**	0.45
EN	**0.59**	**-0.63**	**-0.58**	-0.15	0.09	**-0.62**	0.20	0.31	**0.80**	**0.78**	0.56
NO_3^-	**0.63**	**-0.66**	**-0.65**	-0.19	0.16	**-0.71**	0.29	0.33	**0.82**	**0.80**	0.55
NH_4^+	-0.04	-0.02	0.12	0.09	-0.24	0.04	-0.43	-0.19	0.00	0.13	-0.08
Clay	**0.58**	**-0.60**	**-0.67**	-0.16	0.21	**-0.81**	0.18	0.35	**0.83**	**0.89**	0.43
Sand	0.17	-0.10	-0.12	-0.42	-0.09	0.28	0.13	0.28	-0.12	-0.17	0.05
Silt	**-0.64**	**0.65**	**0.70**	0.22	-0.16	**0.77**	-0.20	-0.39	**-0.81**	**-0.85**	-0.47
Ergosterol	-0.46	0.51	0.24	-0.03	**0.57**	0.39	-0.02	-0.08	-0.45	-0.37	-0.51
Xylanase Q_{10}	0.17	-0.16	0.03	-0.12	-0.31	0.33	0.13	0.15	-0.11	-0.20	0.08

Table S7.2 Continued

Xylanase Q_{10} in Pooled data

	C_{mic}	N_{mic}	EOC	EN	NO_3^-	NH_4^+	Clay	Sand	Silt	Ergosterol
	µg C mg^{-1} SOC	µg N mg^{-1} SOC	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	µg g^{-1} soil	%	%	%	µg mg^{-1} SOC
r_2930										
r_1620										
r_1530										
r_1159										
β-glucosidase										
Xylanase										
pH										
Carbonate										
SOC										
TN										
CN										
C_{mic}										
N_{mic}	**0.72**									
EOC	0.15	0.28								
EN	0.27	0.08	**0.64**							
NO_3^-	0.35	0.20	0.55	**0.95**						
NH_4^+	-0.31	-0.32	0.32	0.20	0.07					
Clay	0.20	0.26	**0.63**	**0.75**	**0.77**	0.15				
Sand	-0.13	0.04	-0.11	-0.17	-0.14	-0.03	-0.10			
Silt	-0.15	-0.25	**-0.58**	**-0.72**	**-0.76**	-0.12	**-0.96**	-0.09		
Ergosterol	0.32	0.12	-0.32	-0.38	-0.37	-0.03	-0.20	0.02	0.22	
Xylanase Q_{10}	-0.05	-0.09	-0.06	-0.12	-0.15	-0.14	-0.24	0.18	0.20	-0.05

Table S7.3 Relative decrease in r^2 like parameter (Pseudo r^2) of the linear mixed effect models calculated by dropping significant variables one by one. Value next to each variable is the absolute change in model r2 after removing the respective variable. Sorting of the variables is according to their ranking of significance.

	Landscape Scale				Regional Scale	
	Kraichgau		Swabian Alb			
	Factors	**Pseudo r^2**	**Factors**	**Pseudo r^2**	**Factors**	**Pseudo r^2**
Soil basal respiration	Complete model	0.58	Complete Model	0.57	Complete model	0.65
	SOC	0.43	C_{mic}	0.13	CN	0.52
	NH_4^+	0.49	*β-gluco. Pot.	0.48	C_{mic}	0.54
	Silt	0.51			Xylanase	0.57
					NO_3^-	0.62
RQ_{10}	Complete model	0.39	Complete model	0.43	Complete model	0.17
	Clay	0.15	EOC	0.27	C_{mic}	0.06
	pH	0.24	Clay	0.27	Silt	0.08
			r_1159	0.29	NO_3^-	0.12
			NH_4^+	0.35	r_1159	0.14
β-glucosidase Q_{10}	Complete model	0.45	Complete model	0.90	Complete model	0.22
	pH	0.21	pH	0.68	β-gluco. Pot.	0.00
	β-gluco. Pot.	0.25	CN	0.73	Ergosterol	0.16
	Ergosterol	0.30	r_1530	0.82		
	SOC	0.36	NH_4^+	0.83		
			r_1159	0.87		
			β-gluco. Pot.	0.87		
			Carbonate	0.87		
			SOC	0.87		
Xylanase Q_{10}	Complete model	0.37	Complete model	0.30	Complete model	0.19
	r_1159	0.22	Xylanase		r_2930	0.01
	β-gluco. Pot.	0.23			Clay	0.07

* β-gluco. Pot. = Potential β-glucosidase activity