Soil enzyme activity and stoichiometry along a gradient of vegetation restoration at the Karst Critical Zone Observatory in southwest China

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Abstract

The ‘Grain for Green Programme (GGP)’ was implemented in the 1990s as a solution to the extreme degradation of karst landscapes that cover one-third of China, largely caused by decades of poorly managed intensive agriculture. The recovery of soil functions is key to the success of ecosystem regeneration of abandoned croplands where the carbon (C) and nutrient cycles have been severely perturbed by cultivation. However, an ecological ‘tipping point’ beyond which soil functions are unrecoverable in manageable timescales may have been passed in the fragile, subtropical karst ecosystem. The aim of this study was to use the activity of key enzymes for C, nitrogen (N) and phosphorus (P) acquisition in the soil as a proxy for the biological response to vegetation restoration after agricultural abandonment in a severely degraded karst catchment at the Karst Critical Zone Observatory in Guizhou Province. In 2016, a space-for-time approach was used to establish a chronosequence of vegetation recovery: sloping cropland < recently abandoned sloping cropland < shrubland < secondary (regenerated) forest < primary (natural) forest. Soils were sampled from the surface to the bedrock (up to 80 cm depth) in each recovery phase. The activity of all enzymes in the top 0-30 cm depth increased after abandonment and was positively correlated with soil nutrient and water contents. Nitrogen deficiencies were indicated by the reduced ratios of C- relative to N-hydrolase activity and the increased ratios of N- relative to P-hydrolase activity in the abandoned croplands and shrublands. Phosphorus deficiencies were indicated by the reduced ratios of N- relative to P-hydrolase activity and C- relative to P-hydrolase activity in the soils of the shrubland and secondary forest compared to the primary forest. Our results revealed that near-to-natural biological soil function was recoverable as vegetation naturally restored and suggested that the rate of recovery may be accelerated by managed nutrient amendments during the early stages after abandonment. This new information may help to inform the managed regeneration of degraded agricultural land in nutrient-poor, sub-tropical environments.

Keywords: soil organic carbon, nitrogen, phosphorus, enzyme activity, vegetation restoration; karst ecosystem

1 INTRODUCTION

Soil degradation caused by human perturbation causes unsustainable losses in net primary productivity (NPP) that threatens human wellbeing and development across the world (Bai et al., 2011). The most recent Global Assessment of Land Degradation and Improvement concluded that land degradation is most conspicuous in the rapidly developing, humid south of China, by comparison with the dry lands of the north and west (Bai et al., 2009). Over the last 50 years, unsustainable intensification of agriculture, exploitation of mineral resources and encroaching urbanization have caused progressive and severe degradation in substantial areas of the fragile karst ecosystems of southwest China, one of the largest karst regions in the world (Montgomery, 2007). The characteristic steeply sloping topographies of karst landscapes have shallow soil profiles that are vulnerable to erosion and leaching (Delang & Yuan, 2015; Quine et al., 2017). In many areas of the karst landscapes of southwest China, a 'tipping point' appears to have been passed as basement rock is exposed and 'rocky desertification' dominates because soil is being lost as a consequence of accelerated rates of erosion caused by human perturbation (Wang et al., 2004). Thus, there is a pressing need to better understand and quantify the changes that occur in soil quality and ecosystem function before this extreme state is reached so that appropriate management stratagies, if any, can be informed (Quine et al., 2017).

In recognition of the severity of the degradation problem in the karst ecosystems of Southwest China, the Chinese Government implemented the 'Grain for Green Programme' (GGP) in the region in the late 1990s. Croplands on slopes greater than 25° were abandoned and vegetative succession allowed to proceed naturally from non-woody plants to woody shrubs and ultimately to mature secondary forest, with the ultimate objective of ecosystem restoration to conditions similar to pristine primary forest (Xu et al., 2006). The regeneration of plant communities under GGP in degraded karst landscapes has been largely successful, indicated by increased net primary productivity (NPP) and soil organic matter (SOM) stocks, and the stabilization of soil profiles by vegetative canopy cover and roots that moderate further loss by erosion (Chang et al., 2017; Ren et al., 2017; Tong et al., 2018). Soil organic matter contains soil organic carbon (C) and nutrients (including nitrogen (N) and phosphorus (P)) and is recognized as a key indicator of soil health and productivity in agricultural systems (Dungait et al., 2012a). The

turnover of SOM and release of nutrients is mediated by the soil microbial community (e.g. bacteria and fungi including mycorrhiza) that also play a role in soil stabilization through the formation of soil aggregates, through the process of 'soil self-organisation' (Young & Crawford, 2004), increasing the potential for successful vegetation colonization and succession by improving soil quality, i.e. nutrient and water retention and supply and diffusion of gases through soil pores (Brahma et al., 2018).

Ecosystem responses to land use change are controlled by the potential to adapt to changing C and nutrient supply, described as 'stoichiometric flexibility' by Sistla & Schimel (2012). Resource availability controls biological processes that subsequently effect further change in supply and acquisition to meet the basic metabolic requirements of all organisms (Elser et al., 1996). Cleveland & Liptzin (2007) recognized constrained atomic C:N:P ratios in soils and the soil microbial biomass at the global scale, that nevertheless showed significant variation in C:N, C:P and N:P ratios between vegetation types, e.g. forest *versus* grassland. This approach has been applied to investigate soil microbial responses to land use change in Chinese karst ecosystems. For example, Li et al. (2012) determined significant variation in the C:N:P ratios of soil and soil microbial biomass in typical karst mountain, low hill and lowland landscapes that had been caused by human perturbation. Hu et al. (2016) described coincident increases in soil and microbial C:N ratios in reforested karst soils that demonstrated an adaptive response by the soil microbial community to changes in substrate resource stoichiometry. Soils in natural karst ecosystems are characteristically nutrient-limited because of underlying geology, and many studies have explored nutrient limitations using N:P ratios of soil (Jiao et al., 2013), plant foliage (Zhang et al., 2015a) and microbial biomass (Tischer et al., 2014). Typical changes in soil N:P ratios caused by a shift from N- to P-limitation during vegetation restoration are observed in karst ecosystems (Zhang et al., 2015a) as N leaches readily in the early stages after abandonment with sparse vegetation coverage (Song et al., 2017), and P adsorbs strongly to calcium (Ca) weathered from the bedrock (Vitousek et al., 2010).

Most studies of soil processes focus on surface soil horizons where soil microorganisms are more abundant in response to greater inputs of organic matter from decomposing surface and root litter and rhizoexudates (Gocke et al., 2017; Peng & Wang, 2016). Soil enzyme activities

decline exponentially with depth in the soil profiles of temperate grasslands and tropical forests (Blume et al., 2002; Stone et al., 2014). However, belowground competition for nutrients and water by plant roots is recognized in many ecosystems, and symbiotic mycorrhizae scavenge throughout the soil in resource-limited soils, e.g. in arable soils after long term cultivation and litter removal (Kautz et al., 2013). In karst systems, the depth of soils is generally considered to be very shallow due to slow rates of pedogenesis from highly soluble bedrock, but the potential for soil accretion in deep geological formations in the epikarst is substantial but poorly understood. The combination of the unique geology of karst ecosystems and the limited understanding of soil enzyme activity and their ratios in karstic subsoils suggests a unique opportunity to understand the role of subsoil nutrient cycling and supply during vegetation restoration in these fragile ecosystems.

Soil microorganisms and some plant roots produce extracellular enzymes that catalyze the mineralization of SOM, releasing nutrients into soil solution that become available for take-up by plant roots (Burns et al., 2013). The resource allocation strategies of soil microorganisms to metabolic processes (including enzyme biosynthesis) respond to the availability of substrates and are constrained by their stoichiometric requirements to maintain metabolic and nutrient balances (Sinsabaugh et al., 2002). Therefore, soil enzyme activity has been used as a sensitive indicator of biological function for several decades (e.g. Dick, 1994) and extracellular enzyme activities are commonly interpreted as indicators of microbial nutrient demand (Sinsabaugh et al., 2008). Soil hydrolases preferentially catalyze nutrient-rich ‘labile’ SOM related directly to meeting the C, N and P nutrient requirements of soil microorganisms and plants, whereas soil oxidase activities are related to the turnover of poor quality ‘recalcitrant’ polyphenolic organic matter, e.g. lignin, terpenoids and humified organic matter (Sinsabaugh & Shah, 2011). Thus, when investigating proximity to a ‘tipping point’ in ecosystem function, or ecosystem regeneration away from a degraded state, the activity of ubiquitous enzymes for C and nutrient cycling in soils should provide a reliable indicator of change in soil biological function. A global scale meta-analysis of 40 terrestrial ecosystems revealed that the ratios of soil lnC: lnN: lnP nutrient acquisition hydrolase activities were close to 1:1:1, indicating the microbial nutrient acquisition capacities were generally similar (Sinsabaugh et al., 2008). Xu et al. (2017)

investigated C, N and P nutrient acquisition hydrolase activities in forests along the North-South Transect in eastern China (NSTEC) using the ratios of lnC:lnN:lnP. They concluded that the reduced ratios of lnC:lnP and lnN:lnP tropical and subtropical forest soils indicated predominant P-limitation compared with temperate forests and the global average. Thus, changes in extracellular enzyme activity and their ratios may be used as a tool to explore the constraints on microbial biomass (Sinsabaugh et al. 2008) by providing a functional assessment of nutrient availability and limitations for vegetation restoration due to inadequate soil fertility (Waring et al., 2014).

The overall objective of this study was to qualify and quantify how the activities and ratios of soil enzymes through soil profiles responded to changes in nutrient availability during vegetative restoration of a degraded watershed under GPP compared to a non-degraded pristine forest in the karst ecosystem of Southwest China. We hypothesized that N would be limiting in the early stages of restoration when the vegetation cover was sparse because of low N inputs and strong nitrate leaching, and soil C:N acquiring hydrolase activities would also be reduced in the early stages compared to later stages of restoration due to larger organic matter input from increasing NPP. We also postulated that soil N:P acquiring hydrolase activities would be poor because karst soils are naturally P-limited due to low inputs of P from parent rock weathering and strong adsorption by Ca.

2 MATERIALS AND METHODS

2.1 Study sites

Two field sites in close proximity with the same soil type and climate regime (Chenqi watershed and Tianlong Mountain) were selected for study at the Karst Critical Zone Observatory in Puding County, Guizhou Province, Southwest China, encompassing five recovery phases along a chronosequence of recovery in the karst ecosystem. The sloping cropland, recently abandoned sloping cropland, shrubland and secondary (regenerated) forest were in the Chenqi watershed, and the primary (pristine) forest was on Tianlong Mountain. The two field sites are representative of the regional karst ecosystem and subject to a subtropical monsoon climate.

The annual mean precipitation was 1315 mm, with an annual mean temperature of ~15.1 °C (Liu et al., 2016). The soil in both areas was dominated by Mollic Inceptisols that originated from the limestone bedrock of the Middle Triassic Guanling Formation (Lu et al., 2014). Table 1 provides the landscape and management characteristics of the sites and the dominant vegetation species on each land use.

The Chenqi catchment (26°15′37″- 26°15′40″ N, 105°46′11″- 105°46′29″ E) ranges in elevation from 1100 and 1600 m above sea level (asl), with an area of 1.29 km^2. Intensive cultivation (predominantly maize, oil seed rape and soybean in rotation with regular application of urea as a single compound or combined with manure) had been practised on the hillslopes since the 1960s. The GGP was introduced here in the 1990s to encourage abandonment of large areas of severely degraded sloping cropland on middle and upper slopes allowing regeneration to woody shrubland (2-3 years) and then secondary forest (10 years +). Tianlong Mountain (26° 14′44″- 26°14′48″N, 105°45′40″- 105°45′46″E) is predominately an undisturbed, pristine primary forest with no history of farming, with an elevation between 1421 and 1503 m asl.

2.2 Soil sampling

In July 2016, we used a space-for-time approach to establish a chronosequence of change following agricultural land abandonment under GGP. Soils were sampled from four replicate 10 m × 20 m plots in each of the five recovery phases: sloping cropland < recently abandoned sloping cropland < shrubland < secondary (regenerated) forest < primary (natural) forest in the Chenqi catchment and primary forest on Tianlong Mountain (i.e. 20 plots in total). A soil auger with a diameter of 2 cm was used to collect soil samples from different soil depths intervals (i.e. 0–10, 10–30, 30–50, 50 cm to the bedrock). Soil depth did not extend below 50 cm in the abandoned croplands and shrublands. To account for spatial heterogeneity, ten soil cores were randomly collected within each plot and composited into a single soil sample. Each composite sample was stored at 4 °C until analysis for soil water content (SWC), pH, and concentrations of dissolved organic carbon (DOC), available nitrogen (AN), available phosphorus (available

187 P) and soil enzyme activities. Subsamples were air dried, homogenized and ground to 0.15 mm
188 for soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) determination.

189 2.3 Soil environmental parameters

190 Percentage SWC was measured by oven drying fresh, sieved (2mm) soil at 105 °C to constant
191 weight (Bao, 2008). Soil pH was measured in a 1:2.5 v:v soil-water suspension using a glass
192 electrode (Bao, 2008). SOC (g kg^{-1}) was determined by dichromate oxidation and titration with
193 ferrous ammonium sulfate (Bao, 2008). TN (g kg^{-1}) was determined by dry combustion using
194 an element analyzer (Elementar, Vario Max CN, Germany) (Bao, 2008). DOC (mg kg^{-1}) was
195 extracted in distilled water at a soil: distilled water ratio of 1:5 and quantified using an organic
196 carbon analyzer (Elementar, Liqui TOC II, Germany) (Bao, 2008). TP (mg kg^{-1}) was measured
197 spectrophotometrically with a continuous flow auto-analyzer (Bran Luebbe, AA3, Germany)
198 after digestion with H_2SO_4-$HClO_4$ (Bao, 2008). AN (mg kg^{-1}) was determined after extraction
199 with 1 mol L^{-1} KCl, and available P after extraction with 0.5 mol L^{-1} $NaHCO_3$, by continuous
200 flow analyser (Bran Lubbe, AA3, Germany) (Bao, 2008).

201 **2.4** Enzyme assays

202 The potential activities of three C-acquiring enzymes (β-1,4-glucosidase, βG, EC 3.2.1.21; β-
203 D-1,4-cellobiosidase, CBH, EC 3.2.1.91; β-1,4-xylosidase, βX, EC 3.2.1.37), two N-acquiring
204 enzymes (β-N-acetyl glucosaminidase, NAG, EC 3.1.6.1; leucine aminopeptidase, LAP, EC
205 3.4.11.1) and one P-acquiring enzyme (alkaline phosphatase, AP, EC 3.1.3.2) in the soil
206 samples were measured following a modified fluorescence method (German et al., 2011; Trap
207 et al., 2012). In brief, 1 g of fresh soil was homogenized in 125 ml 2-morpholin-4-
208 ylethanesulfonic acid (MES) buffer (pH = 6.5) or 2-Amino-2-(hydroxymethyl) propane-1,3-
209 diol (Tris) buffer (pH = 7.5). The pH of the buffers was selected because they were similar to
210 the soil pH, according to German et al. (2011). 200 µl of homogenate and 50 µl of substrate
211 was added to a 96-well black microplate. Eight replicates for each soil sample, blanks, negative
212 controls and quench standards were analyzed. The microplates were incubated in the dark at
213 25 °C for 4 hours according to Saiya-Cork et al. (2002). After incubation, 10 µl of 1 mol L^{-1}
214 NaOH was added to each well to terminate the reactions, and fluorescence values were

measured using a microplate fluorometer (Synergy^{H4}, BioTek, USA) with excitation and emission filters of 365 and 450 nm, respectively. The absolute hydrolase activities were expressed in units of nmol g^{-1} soil h^{-1} after correcting for negative controls and quenching. The ratios of C, N and P nutrient acquiring hydrolase activities were compared using lnBG:ln(NAG+LAP):lnAP.

Soil oxidase activities (i.e. Peroxidase, POD, EC 1.10.3.2; Polyphenol oxidase, PPO, EC 1.11.1.7) were assayed colourimetrically using L-3,4-dihydroxyphenylalanine (L-DOPA) following a modified microplate protocol optimized for *in situ* pH conditions (German et al., 2011; Saiya-Cork et al., 2002). In brief, 600 µl of homogenate and 150 µl of substrate were added to deep 96 well microplates. For measuring the POD activities, an extra 10 µl of 0.3% H_2O_2 was added, then incubated for 5 hours at 25°C in the dark. After incubation, the microplates were centrifuged at 3000 rpm for 3 minutes, and 250 µl of liquid supernatant was transferred to a 96 well transparent microplate. The absorbance values were measured at 460 nm by microplate spectrophotometer (Synergy^{H4}, BioTek, USA). The absolute oxidase activities were expressed in units of µmol g^{-1} soil h^{-1}. The substrates and functions of the enzymes are summarized in Table S1.

2.5 Statistical analyses

All results are reported as means ± standard errors, and analysis was completed using SPSS 21 software (IBM SPSS Statistics, USA). All variables of data were tested for normal distribution and homogeneity of variance, with no transformations necessary. Two-way ANOVA was used to identify the significant differences in the activities and stoichiometry of C, N, and P nutrient-acquiring enzymes and the soil properties of different soil depths among land use types. Duncan's test was used for Post-Hoc comparisons to identify the significant sub-sets, if apparent. The relationships between the soil properties and the soil enzyme ratios were assessed using Pearson correlations in SPSS. Results were deemed statistically significant when $p < 0.05$. The figures were plotted in OriginPro 2017 (Originlab Corporation, USA).

3 RESULTS

3.1 Soil properties

Soil moisture, SOC and TN contents were significantly affected by land use types, soil depth and their interactions (Table 2, S2, S3, S4, $p < 0.05$). The SWC (%) was larger in the top 30 cm of soil from the secondary (+61%, $p < 0.05$) and primary forests (+50%, $p < 0.05$) compared to the sloping croplands. The SOC (+119%, $p < 0.05$) and TN contents (+70%, $p < 0.05$) were greater in the secondary forests than in the sloping croplands, and ~184% and 150% larger in the primary forests compared to the sloping croplands respectively ($p < 0.05$). The DOC contents were increased in the 0-10 cm soil depth from the shrublands (+181%, Table 2, S3, $p < 0.05$) and secondary (+73%, $p < 0.05$) and primary forests (+185%, $p < 0.05$) relative to the abandoned croplands. The AN content was increased in the 0-10 cm soil depth from the shrublands (+104%, $p < 0.05$) and secondary (+141%, $p < 0.05$) and primary forests (+193%, $p < 0.05$) compared to the abandoned croplands.

TP contents were significantly decreased in the shrublands and secondary forests compared to the other vegetation types, and available P contents were significantly larger in the primary forests than in the other vegetation types (Table S3, $p < 0.05$). The SOC:TN, SOC:TP and TN:TP ratios were increased in the shrublands and secondary forests relative to the other land use types throughout the soil profile (Table S3, $p < 0.05$).

Along the soil profiles, total nutrient contents (i.e. SOC, TN, and TP) declined with depth (Table S4, $p < 0.05$). The AN contents did not vary along the soil profiles in the abandoned croplands and shrublands (Table 2, $p < 0.05$). The SWC was greater in the top 10 cm compared to below 10 cm in the secondary and primary forests ($p < 0.05$).

3.2 Soil enzyme activity and their ratios

The results of two-way ANOVA indicated that soil depth, land use type and their interactions could all influence soil hydrolase and oxidase activities (Fig. 1a-f, Table S2, $p < 0.05$). The soil hydrolase activities were affected by the land use type at depths of 0-30 cm. Moreover, the BG, BX and CBH activities were approximately 3.6, 2.0 and 2.3 times increased, respectively, in the primary forest compared to the abandoned cropland (Fig. 1a, b, c, $p < 0.05$). The NAG, AP and LAP activities in the primary forests were 11.9, 4.1 and 1.0 times larger, respectively, than

in the abandoned croplands (Fig. 1d, e, f, $p < 0.05$). The soil PPO and POD activities in the 0-30 cm soil layers were greater in the secondary and primary forests relative to the other land use types (Fig. 1g, h, $p < 0.05$).

The results of two-way ANOVA indicated that soil depth and land use type influenced soil enzyme activity ratios (Fig. 2, 3). The average ratio of soil lnBG:ln(NAG+LAP):lnAP among the land use types at the four depth intervals was 1:1.2:1.4. The ratios of soil lnBG:ln(NAG+LAP) were less in the abandoned croplands (0.73) and shrublands (0.74) compared to the other land uses (0.82~0.84, Fig. 2a, $p < 0.05$). The ratios of ln(NAG+LAP):lnAP were increased in the abandoned croplands (0.84) compared to the other land uses (0.70~0.78; Fig. 2b, $p < 0.05$). The ratios of lnBG:lnAP were reduced in the shrublands (0.55) relative to the other land use types (0.60~0.64; Fig. 2c, $p < 0.05$).

The soil hydrolase and oxidase (PPO and POD) activities decreased in the top 30 cm of soil but did not differ below 30 cm (Fig. 1a-h, Table S4). Soil NAG, LAP, and AP activities decreased with soil depth in the secondary and primary forests, but not in the other land uses types (Fig. 1d, e, f, $p < 0.05$). Soil lnBG:lnAP ratios were greater in the top 30 cm than below 30 cm (Fig. 3, $p < 0.05$).

3.3 Correlations between the soil enzyme activities and soil properties

The results of Pearson correlation analysis showed that soil hydrolase and oxidase activities, and their stoichiometric relationships (i.e. lnBG:ln(NAP+LAP) and lnBG:lnAP) were generally positively related to SWC, SOC, DOC, TN, AN and TP. In addition, soil hydrolase activities were positively related to available P, SOC:TN and SOC:TP. The ratio of lnBG:ln(NAP+LAP) was negatively correlated with pH, while lnBG:lnAP was positively correlated to SOC:TN(Table 3, $p<0.05$).

4 DISCUSSION

4.1 Soil enzyme activity in soil profiles at different stages of vegetation recovery

The synthesis of enzymes by microorganisms in response to environmental stress associated

298 with land management change is likely to be rapid (Burns et al., 2013). In some instances,
299 changes in microbial populations or activity can precede detectable changes in soil physical and
300 chemical properties, thereby providing an early sign of soil improvement or an early warning
301 of soil degradation (Pankhurst et al. 1995). In this study, the differences between vegetation
302 recovery stages (i.e. sloping farmland, abandoned farmland, shrubland, secondary forest and
303 primary forest) were clear across all of the soil enzymes considered in this study (see Fig. 1).
304 Soil C, N, and P nutrient-acquisition enzyme activities were all significantly positively
305 correlated with total soil nutrients (i.e. SOC, TN, and TP) and available nutrient content (i.e.
306 DOC, AN and available P) as expected in nutrient-limited karst ecosystems (Xu et al., 2015;
307 Ren et al. 2016). The relationship between vegetation restoration and associated increases in
308 NPP and SOM content are widely recognised (e.g. Liu et al., 2015) because there is a direct
309 enhancement of the supply of substrate to soil microorganisms, and an indirect increase in
310 supply via elevated soil hydrolase activity (i.e. positive feedback). As expected, and consistent
311 with previous studies (e.g. Peng & Wang, 2016; Stone et al., 2014), land use type had the
312 predominant effect on the soil enzyme activities in the top 30 cm of the soil profile where input
313 from plant litter and roots is greatest and nutrients are most abundant (Jackson et al., 1996; Lee
314 et al., 2014), compared to below 30 cm depth where SOM content is reduced and of poorer
315 quality and oxygen supply may be limiting (Fontaine et al., 2007; Schrumpf et al., 2013; Bai et
316 al., 2015).

317 A positive relationship between *in situ* SWC and soil enzyme activity observed herein (Table
318 3) supports the widely reported importance of soil moisture for biological function, including
319 soils in the Chinese karst ecosystem (Chen et al. 2017). It is likely that this effect was a product
320 of enhanced water holding capacity associated with increasing organic matter inputs from
321 greater litter inputs above- and below-ground (roots and rhizoexudation) as NPP increased
322 along the recovery gradient from arable to secondary forest, combined with the enhanced
323 abundance and activity of soil microorganisms and subsequent improvements in soil
324 aggregation (Zhu et al., 2012; Wang et al, 2015). Soil aggregation improves soil pore
325 connectivity through an increase in soil macropores, providing pathways for water, nutrients
326 and air that supports biological function, including root proliferation. Enzyme biosynthesis and

secretion is subsequently enhanced as the soil microbial community proliferates under more favourable physical and chemical conditions (Keeler et al., 2009). We recognize that plant roots in the study plots may also secrete enzymes directly into the soil to acquire nutrients (Dakora & Phillips, 2002), but we did not differentiate between sources in this study.

In the sloping croplands, soil hydrolase and oxidase activities in the 0-30 cm depth were generally relatively increased in the actively cultivated soils prior to abandonment under GPP. We suggest that the regular, largely manual tillage (by hoe), fertilizer application and crop residues increased the porosity of the top soil horizon directly, leading to an enhancement of aerobic enzyme activity (Bandick & Dick, 1999; Munkholm et al., 2016). By contrast, the recently abandoned croplands may have experienced a rapid diminishment in nutrient availability, fast decomposition of crop residues and SOM (i.e. priming; Kuzyakov et al., 2000), and soil compaction caused by slaking (i.e. collapse of soil structure by water inundation) in the recently abandoned croplands. Furthermore, the application of organic manures may cause a short-lived increase in enzyme activity from inputs of faecal bacteria in fresh animal dung, or by promoting the secretion of enzymes by Gram positive bacteria, including Actinobacteria, in particular (Zhang et al., 2015b). In this study, soil oxidase and hydrolase activities were observed to display coincident activity as vegetative succession progressed, associated with increasing decomposition of greater organic matter inputs from larger NPP, which was consistent with the results of Zhang et al., (2012). Soil hydrolases and oxidases often co-occur in the same vegetation type (Baldrian, 2014), because (1) the consumption of peroxide and phenolic substances by oxidases may alleviate the inhibitory effects of phenolic molecules on soil enzymes and microorganisms (Sinsabaugh, 2010), which may, in turn, promote soil hydrolase activities; and (2) soil oxidative enzymes may also increase substrate supply for hydrolase activity by degrading complex polymeric organic matter, which could promote further increases in soil hydrolase activities and nutrient contents (i.e. positive feedback) (Tian & Shi, 2014). Therefore, intensified oxidase activities may indicate increased biological availability of nutrients in secondary and primary forests that promotes further microbial activity (Li et al., 2018). The increased SOC and DOC contents in the secondary and primary forests (Table 3) may trigger soil oxidase activities as SOC and DOC supply energy to facilitate

the energy-intensive process of soil oxidase degradation (Schimel & Weintraub, 2003). Increased inputs of complex phenolic substances, i.e. lignin and suberin, may promote increases in oxidase activity in secondary and primary forests (Nannipieri et al., 2012).

Enzyme activities were reduced below 30 cm compared to the top 30 cm of the soil profile, which reflects the lack of biologically available C and nutrients in deeper soils horizons (Fontaine et al., 2007; Schrumpf et al., 2013) and reduced porosity and pore connectivity which hinders the diffusion of water, enzymes and substrates and oxygen (Dungait et al., 2012b; Bai et al., 2015). Furthermore, and consistent with previous studies (e.g. Peng & Wang, 2016; Stone et al., 2014), land use (vegetation) type had a predominant effect on the soil enzyme activities in the top 30 cm of the soil profile, corresponding to changes in the distribution of litter, roots and SOM (Jackson et al., 1996; Lee et al., 2014). A meta-analysis of the root distribution in all ecosystems averaged worldwide showed that approximately 65% of the plant roots were distributed in the top 30 cm of the soil profile (Jackson et al., 1996). Roots supply nutrients through root turnover, rhizoexudation and the creation of semi-permanent soil pores allowing improved infiltration of water, nutrients and oxygen from the soil surface. This is disrupted in intensively managed agricultural systems through tillage and harvest including removal of both surface residues and roots.

4.2 The ratios of soil enzyme activities at different stages of vegetation restoration in the soil profile.

Soil enzyme ratios provide proxy information about nutrient availability to soil microorganisms and other limiting factors of the ecosystem. The C:N:P acquisition ratio 1:1.2:1.4 measured by lnBG:ln(NAG+LAP):lnAP was less than the global and regional averages (1:1:1) reported by Sinsabaugh et al. (2008), which indicates that soil N and P nutrient availabilities in this subtropical karst ecosystem were generally poorer than the global average. The cropland and post-abandonment land use types were all deficient in P (TP < 400 mg kg^{-1} and available P < 4 mg kg^{-1}) and N (AN < 60 mg kg^{-1}) according to the classification values for Chinese soils (National Soil Survey Office, 1998; Yang et al., 2014).

In agreement with our first hypothesis, and the findings of Zhang et al. (2015b), the lower ratios of lnBG:ln(NAG+LAP) and the higher ratios of ln(NAG+LAP):lnAP indicated N-deficiency predominated in the abandoned croplands and shrublands, which is also suggested by the positive relationship indicated by lnBG:ln(NAG+LAP) and TN or AN (Table 3). Zhang et al. (2015a) had also observed N-limitation during early phases of vegetation restoration in karst ecosystems using changes in foliage N:P ratios. Soil N can recover relatively quickly after initial rapid losses following cessation of N-fertilizer application during agricultural abandonment if weathering of N-rich bedrock proceeds rapidly, or atmospheric N deposition from local industrial/urban emissions are abundant, or biological N fixation (free-living or rhizobial) develops (Wen et al., 2016, Li et al., 2018). However, N-loss from karstic soils through runoff and leaching may be relatively rapid because of the shallow soil depth, and potential for accelerated losses through the porous bedrock and the epikarst that maintains soil N deficiencies in the long term (Song et al., 2017). Subsequently, we observed that the poor AN did not change within the soil profiles, coincident with an increase in N-acquiring hydrolase activities because N was scarce, in accordance with enzyme economic theory (Sinsabaugh et al., 2002).

We also hypothesized that the productivity of soils in karst ecosystems would be P-limited because of the paucity of the element in the parent rock and strong adsorption to Ca minerals. We observed that the lnBG:lnAP and ln(NAG+LAP):lnAP ratios were less than the global average reported by Sinsabaugh et al. (2008), indeed indicating P deficiency in the abandoned croplands, shrublands, and secondary forests. In addition, inorganic P applications to sloping croplands may inhibit soil AP activities (Zhang et al., 2015b). By contrast, AP activity in the primary forests was decreased relative to C-acquiring enzyme activities in the primary forest at Tianlong Mountain. Unlike P-limitation observed in the primary forest by Zhang et al. (2015a), we suggest that the natural forest ecosystem studied herein was well-adapted to P-deficiency through the development of mutualistic relationships between trees and arbuscular mycorrhizal fungi, for example (Liang et al., 2016). The larger soil microbial biomass in undisturbed primary forests in karst ecosystems (Zhu et al., 2012), relative to grasslands or shrublands, is likely adapted to alleviate P limitation by releasing hydrolase enzymes to catalyze SOM

413 decomposition and promote nutrient recycling. Furthermore, rhizoexudates, including organic
414 acids, are more abundant in forests than shrublands (Jackson et al., 1996), increasing the
415 potential for the acquisition of P bound to Ca minerals and from SOM (Pan et al., 2016). the TP
416 and available P contents of the sloping and abandoned croplands, shrublands and secondary
417 forests were considered 'deficient' with respect to the classification of soil P contents across
418 China (National Soil Survey Office, 1998; Yang et al. 2014), i.e. class 5, soil TP (200-400 mg
419 kg^{-1}) and available P content (3-5 mg kg^{-1}). From the perspective of nutrient ratios, in addition,
420 the soil C:P and N:P ratios indicated that the P deficiency was more severe in the shrublands
421 and secondary forests than in the other land use types (Tian et al., 2010). In the abandoned
422 croplands, the available P concentrations were relatively poor, which might indicate that the
423 measured variations in nutrient stoichiometry were a less sensitive indicator of biological
424 nutrient availability than soil enzyme ratios.

425

426 **5 CONCLUSIONS**

427 In fragile and nutrient-limited karst ecosystems, vegetation restoration after agricultural
428 abandonment caused changes in soil enzyme activities in the top 0-30 cm soil depth, indicating
429 that the nutrient (C, N and P) acquisition abilities of soil microorganisms adapted to the
430 changing soil environment at each stage of recovery towards climax vegetation (forest).
431 Oxidase activities, soil nutrient and soil water contents indicated improved nutrient availability
432 and soil quality in the secondary forests, which was similar to the primary forests. The
433 lnBG:ln(NAG+LAP) and lnBG:lnAP ratios indicated that soil N was deficient in the abandoned
434 croplands and shrublands, and that soil P was deficient in the shrublands and secondary forests.
435 These results suggest that soil microorganisms in karst systems have the potential to adapt to
436 nutrient limitations and subsequently alleviate nutrient limitations to vegetation through their
437 scavenging activities, including symbiotic relationships with mycorrhiza. However, we suggest
438 that ecosystem recovery might be accelerated by the judicious application and monitoring of N
439 and P by directly alleviating nutrient limitation as part of a national strategy to rejuvenate
440 degraded and nutrient limited karst ecosystems in southwest China under GPP.

441

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450 **CONFLICT OF INTEREST STATEMENT**

451 The authors declare no conflicts of interest.

452 **REFERENCES**

453

454 Bai, Z., & Dent, D. (2009). Recent land degradation and improvement in China. *AMBIO: A*
455 *Journal of the Human Environment, 38*, 50-157. https://doi.org/10.1579/0044-7447-
456 38.3.150

457 Bai, Z.G., de Jong, R. & van Lynden, G.W.J. (2011). An update of GLADA - Global assessment
458 of land degradation and improvement. ISRIC report 2010/8, Wageningen: ISRIC - World
459 Soil Information.

460 Bai, R., Xi, D., He, J. Z., Hu, H. W., Fang, Y. T., & Zhang, L. M. (2015). Activity, abundance
461 and community structure of anammox bacteria along depth profiles in three different
462 paddy soils. *Soil Biology & Biochemistry, 91*, 212-221.
463 https://doi.org/10.1016/j.soilbio.2015.08.040

464 Baldrian, P. (2014). Distribution of Extracellular Enzymes in Soils: Spatial Heterogeneity and
465 Determining Factors at Various Scales. *Soil Science Society of America Journal, 78*, 11-
466 18. http://doi.org/10.2136/sssaj2013.04.0155dgs

467 Bandick, A. K., & Dick, R. P. (1999). Field management effects on soil enzyme activities. *Soil*

Biology & Biochemistry, 31, 1471-1479. https://doi.org/10.1016/S0038-0717(99)00051-6

Bao, S. D. 2008. Soil and agricultural chemistry analysis (3rd edn). Beijing: China Agriculture Press. (in Chinese)

Blume, E., Bischoff, M., Reichert, J. M., Moorman, T., Konopka, A., & Turco, R. F. (2002). Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. *Applied Soil Ecology, 20*, 171-181. https://doi.org/10.1016/S0929-1393(02)00025-2

Brahma, B., Pathak, K., Lal, R., Kurmi, B., Das, M., Nath, P. C., . . . Das, A. K. (2018). Ecosystem carbon sequestration through restoration of degraded lands in Northeast India. *Land Degradation & Development, 29*, 15-25. https://doi.org/10.1002/ldr.2816

Burns, R. G., DeForest, J. L., Marxsen, J., Sinsabaugh, R. L., Stromberger, M. E., Wallenstein, M. D., . . . Zoppini, A. (2013). Soil enzymes in a changing environment: Current knowledge and future directions. *Soil Biology & Biochemistry, 58*, 216-234. https://doi.org/10.1016/j.soilbio.2012.11.009

Chang, X. F., Chai, Q. L., Wu, G. L., Zhu, Y. J., Li, Z. W., Yang, Y. F., & Wang, G. J. (2017). Soil organic carbon accumulation in abandoned croplands on the Loess Plateau. *Land Degradation & Development, 28*, 1519-1527. https://doi.org/10.1002/ldr.2679

Chen, H., Luo, P., Wen, L., Yang, L. Q., Wang, K. L., & Li, D. J. (2017). Determinants of soil extracellular enzyme activity in a karst region, southwest China. *European Journal of Soil Biology, 80*, 69-76. https://doi.org/10.1016/j.ejsobi.2017.05.001

Cleveland, C.C., & Liptzin, D. (2007). C: N: P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? *Biogeochemistry, 85*, 235-252. https://doi.org/10.1007/s10533-007-9132-0

Dakora, F. D., & Phillips, D. A. (2002). Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant and Soil, 245*, 35-47. https://doi.org/10.1023/A:1020809400075

de Medeiros, E. V., Duda, G. P., dos Santos, L. A. R., Lima, J. R. D., de Almeida-Cortez, J. S., Hammecker, C., . . . Cournac, L. (2017). Soil organic carbon, microbial biomass and enzyme activities responses to natural regeneration in a tropical dry region in Northeast

497 Brazil. *Catena, 151*, 137-146. https://doi.org/10.1016/j.catena.2016.12.012
498 Delang, C. O., & Yuan, Z. (2015). China's Grain for Green Program. Cham, Switzerland: Springer
499 International Publishing.
500 Dick, R. P. 1994. Soil enzyme-activities as indicators of soil quality. Pages 107-124. In Doran, J.
501 W., Coleman, D. C., Bezdicek, D. F., & Stewart, B. A., editors (Eds.). Defining Soil Quality
502 for a Sustainable Environment. Madison: Soil Science Society of America.
503 Dungait, J. A. J., Cardenas, L. M., Blackwell, M. S. A., Wu, L., Withers, P. J. A., Chadwick,
504 D. R., . . . Goulding, K. W. T. (2012a). Advances in the understanding of nutrient dynamics
505 and management in UK agriculture. *Science of the Total Environment, 434*, 39-50.
506 https://doi.org/10.1016/j.scitotenv.2012.04.029
507 Dungait J. A. J., Hopkins D. W., Gregory A. S., Whitmore A. P. (2012b). Soil organic matter
508 turnover is governed by accessibility not recalcitrance. *Global Change Biology, 18*, 1781-
509 1796. https://doi.org/10.1111/j.1365-2486.2012.02665.x
510 Elser, J.J., Dobberfuhl, D.R., MacKay, N.A., & Schampel, J.H. (1996). Organism size, life
511 history, and N: P stoichiometry. *BioScience, 46*, 674-684. https://doi.org/10.2307/1312897
512 Fontaine, S., Barot, S., Barre, P., Bdioui, N., Mary, B., & Rumpel, C. (2007). Stability of
513 organic carbon in deep soil layers controlled by fresh carbon supply. *Nature, 450*, 277-
514 U210. https://doi.org/10.1038/nature06275
515 German, D. P., Weintraub, M. N., Grandy, A. S., Lauber, C. L., Rinkes, Z. L., & Allison, S. D.
516 (2011). Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies.
517 *Soil Biology & Biochemistry, 43*, 1387-1397.
518 https://doi.org/10.1016/j.soilbio.2011.03.017
519 Gocke, M. I., Huguet, A., Derenne, S., Kolb, S., Dippold, M. A., & Wiesenberg, G. L. B.
520 (2017). Disentangling interactions between microbial communities and roots in deep
521 subsoil. *Science of the Total Environment, 575*, 135-145.
522 https://doi.org/10.1016/j.scitotenv.2016.09.184
523 Hoang, D. T. T., Pausch, J., Razavi, B. S., Kuzyakova, I., Banfield, C. C., & Kuzyakov, Y.
524 (2016). Hotspots of microbial activity induced by earthworm burrows, old root channels,
525 and their combination in subsoil. *Biology and Fertility of Soils, 52*, 1105-1119.

https://doi.org/10.1007/s00374-016-1148-y

Hu, N., Li, H., Tang, Z., Li, Z., Li, G., Jiang, Y., Hu, X., & Lou, Y. (2016). Community size, activity and C: N stoichiometry of soil microorganisms following reforestation in a Karst region. *European Journal of Soil Biology, 73*, 77-83. https://doi.org/10.1016/j.ejsobi.2016.01.007

Jiang, J. P., Xiong, Y. C., Jiang, H. M., Ye, D. Y., Song, Y. J., & Li, F. M. (2009). Soil microbial activity during secondary vegetation succession in semiarid abandoned lands of Loess Plateau. *Pedosphere, 19*, 735-747. https://doi.org/10.1016/S1002-0160(09)60169-7

Jiao, F., Wen, Z. M., An, S. S., & Yuan, Z. (2013). Successional changes in soil stoichiometry after land abandonment in Loess Plateau, China. *Ecological Engineering, 58*, 249-254. https://doi.org/10.1016/j.ecoleng.2013.06.036

Kautz, T., Amelung, W., Ewert, F., Gaiser, T., Horn, R., Jahn, R., . . . Kopke, U. (2013). Nutrient acquisition from arable subsoils in temperate climates: A review. *Soil Biology & Biochemistry, 57*, 1003-1022. https://doi.org/10.1016/j.soilbio.2012.09.014

Keeler, B. L., Hobbie, S. E., & Kellogg, L. E. (2009). Effects of Long-Term Nitrogen Addition on Microbial Enzyme Activity in Eight Forested and Grassland Sites: Implications for Litter and Soil Organic Matter Decomposition. *Ecosystems, 12(1)*, 1-15. https://doi.org/10.1007/s10021-008-9199-z

Kuzyakov, Y., Friedel, J. K., & Stahr, K. (2000). Review of mechanisms and quantification of priming effects. *Soil Biology & Biochemistry, 32*, 1485-1498. https://doi.org/10.1016/S0038-0717(00)00084-5

Lee, H., Fitzgerald, J., Hewins, D. B., McCulley, R. L., Archer, S. R., Rahn, T., & Throop, H. L. (2014). Soil moisture and soil-litter mixing effects on surface litter decomposition: A controlled environment assessment. *Soil Biology & Biochemistry, 72*, 123-132. https://doi.org/10.1016/j.soilbio.2014.01.027

Li, Y., Wu, J., Liu, S., Shen, J., Huang, D., Su, Y., Wei, W., & Syers, J.K. (2012). Is the C: N: P stoichiometry in soil and soil microbial biomass related to the landscape and land use in southern subtropical China? *Global Biogeochemical Cycles, 26*, GB4002. https://doi.org/10.1029/2012GB004399

555 Li, D.D., Zhang, X.Y., Green, S.M., Dungait, J.A.J., Wen, X.F., Tang, Y.Q., . . . Quine. T.A.
556 (2018). Nitrogen functional gene activity in soil profiles under progressive vegetative
557 recovery after abandonment of agriculture at the Puding Karst Critical Zone Observatory,
558 SW China. *Soil Biology and Biochemistry*, *125*, 93–102.
559 https://doi.org/10.1016/j.soilbio.2018.07.004
560 Liang, Y. M., Pan, F. J., He, X. Y., Chen, X. B., & Su, Y. R. (2016). Effect of vegetation types
561 on soil arbuscular mycorrhizal fungi and nitrogen-fixing bacterial communities in a karst
562 region. *Environmental Science and Pollution Research*, *23*, 18482-18491.
563 https://doi.org/10.1007/s11356-016-7022-5
564 Liu, C. C., Liu, Y. G., Guo, K., Zhao, H. W., Qia, X. G., Wang, S. J., . . . Cai, X. L. (2016).
565 Mixing litter from deciduous and evergreen trees enhances decomposition in a subtropical
566 karst forest in southwestern China. *Soil Biology & Biochemistry, 101*, 44-54.
567 https://doi.org/10.1016/j.soilbio.2016.07.004
568 Liu, S., Zhang, W., Wang, K., Pan, F., Yang, S., & Shu, S. (2015). Factors controlling
569 accumulation of soil organic carbon along vegetation succession in a typical karst region
570 in Southwest China. *Science of the Total Environment, 521-522*, 52-58.
571 https://doi.org/10.1016/j.scitotenv.2015.03.074
572 Lu, X. Q., Toda, H., Ding, F. J., Fang, S. Z., Yang, W. X., & Xu, H. G. (2014). Effect of
573 vegetation types on chemical and biological properties of soils of karst ecosystems.
574 *European Journal of Soil Biology, 61*, 49-57. https://doi.org/10.1016/j.ejsobi.2013.12.007
575 Montgomery, D. R. (2007). Soil erosion and agricultural sustainability. *Proceedings of the*
576 *National Academy of Sciences of the United States of America, 104*, 13268-13272.
577 https://doi.org/10.1073/pnas.0611508104
578 Munkholm, L. J., Heck, R. J., Deen, B., & Zidar, T. (2016). Relationship between soil aggregate
579 strength, shape and porosity for soils under different long-term management. *Geoderma*,
580 *268*, 52-59. https://doi.org/10.1016/j.geoderma.2016.01.005
581 Nannipieri, P., Giagnoni, L., Renella, G., Puglisi, E., Ceccanti, B., Masciandaro, G., . . .
582 Marinari, S. (2012). Soil enzymology: classical and molecular approaches. *Biology and*
583 *Fertility of Soils*, *48*, 743-762. https://doi.org/10.1007/s00374-012-0723-0

National Soil Survey Office. 1998. Chinese Soil. Beijing: China Agriculture Press. (in Chinese)

Pan, F. J., Liang, Y. M., Zhang, W., Zhao, J., & Wang, K. L. (2016). Enhanced nitrogen availability in karst ecosystems by oxalic acid release in the rhizosphere. *Frontiers in Plant Science, 7*, 687. https://doi.org/10.3389/fpls.2016.00687

Pankhurst, C. E., Hawke, B. G., Mcdonald, H. J., Kirkby, C. A., Buckerfield, J. C., Michelsen, P., … & Doube, B.M. (1995). Evaluation of soil biological properties as potential bioindicators of soil health. *Australian Journal of Experimental Agriculture, 35*, 1015-1028. https://doi.org/10.1071/EA9951015

Peng, X. Q., & Wang, W. (2016). Stoichiometry of soil extracellular enzyme activity along a climatic transect in temperate grasslands of northern China. *Soil Biology & Biochemistry, 98*, 74-84. https://doi.org/10.1016/j.soilbio.2016.04.008

Quine, T., Guo, D., Green, S. M., Tu, C., Hartley, I., Zhang, X., . . . Liu, H. (2017). Ecosystem service delivery in Karst landscapes: anthropogenic perturbation and recovery. *Acta Geochimica, 36*, 416-420. https://doi.org/10.1007/s11631-017-0180-4

Ren, C. J., Kang, D., Wu, J. P., Zhao, F. Z., Yang, G. H., Han, X. H., . . . Ren, G. X. (2016). Temporal variation in soil enzyme activities after afforestation in the Loess Plateau, China. *Geoderma, 282*, 103-111. https://doi.org/10.1016/j.geoderma.2016.07.018

Ren, Y. J., Lu, Y. H., Fu, B. J., & Zhang, K. (2017). Biodiversity and ecosystem functional enhancement by forest restoration: A meta-analysis in China. *Land Degradation & Development, 28*, 2062-2073. https://doi.org/10.1002/ldr.2728

Rumpel, C., Chabbi, A., & Marschner, B. (2012). Carbon storage and sequestration in subsoil horizons: Knowledge, gaps and potentials. In R. Lal, K. Lorenz, R. F. Hüttl, B. U. Schneider, & J. von Braun (Eds.), *Recarbonization of the Biosphere: Ecosystems and the Global Carbon Cycle* (pp. 445-464). Dordrecht: Springer Netherlands.

Saiya-Cork, K. R., Sinsabaugh, R. L., & Zak, D. R. (2002). The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. *Soil Biology & Biochemistry, 34*, 1309-1315. https://doi.org/10.1016/S0038-0717(02)00074-3

Schimel, J. P., & Weintraub, M. N. (2003). The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biology &*

Biochemistry, 35, 549-563. https://doi.org/10.1016/S0038-0717(03)00015-4

Schrumpf, M., Kaiser, K., Guggenberger, G., Persson, T., Kogel-Knabner, I., & Schulze, E. D. (2013). Storage and stability of organic carbon in soils as related to depth, occlusion within aggregates, and attachment to minerals. *Biogeosciences, 10*, 1675-1691. https://doi.org/10.5194/bg-10-1675-2013

Sinsabaugh, R. L. (2010). Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biology & Biochemistry, 42*, 391-404. https://doi.org/10.1016/j.soilbio.2009.10.014

Sinsabaugh, R. L., Carreiro, M. M., & Repert, D. A. (2002). Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. *Biogeochemistry, 60(1)*, 1-24. https://doi.org/10.1023/A:1016541114786

Sinsabaugh, R. L., Lauber, C. L., Weintraub, M. N., Ahmed, B., Allison, S. D., Crenshaw, C., . . . Zeglin, L. H. (2008). Stoichiometry of soil enzyme activity at global scale. *Ecology Letters, 11*, 1252-1264. https://doi.org/10.1111/j.1461-0248.2008.01245.x

Sinsabaugh, R. L., & Shah, J. J. F. (2011). Ecoenzymatic stoichiometry of recalcitrant organic matter decomposition: the growth rate hypothesis in reverse. *Biogeochemistry, 102*, 31-43. http://doi.org/10.1007/s10533-010-9482-x

Sistla, S.A., & Schimel, J.P. (2012). Stoichiometric flexibility as a regulator of carbon and nutrient cycling in terrestrial ecosystems under change. *New Phytologist, 196*, 68-78. https://doi.org/10.1111/j.1469-8137.2012.04234.x

Song, X., Gao, Y., & Green, M. (2017). Nitrogen loss from karst area in China in recent 50 years: An in-situ simulated rainfall experiment's assessmen. *Ecology and Evolution, 7*, 10131-10142. https://doi.org/10.1002/ece3.3502

Stone, M. M., DeForest, J. L., & Plante, A. F. (2014). Changes in extracellular enzyme activity and microbial community structure with soil depth at the Luquillo Critical Zone Observatory. *Soil Biology and Biochemistry, 75*, 237-247. https://doi.org/10.1016/j.soilbio.2014.04.017

Tian, H. Q., Chen, G. S., Zhang, C., Melillo, J. M., & Hall, C. A. S. (2010). Pattern and variation of C:N:P ratios in China's soils: a synthesis of observational data. *Biogeochemistry*, 98, 139-151. https://doi.org/10.1007/s10533-009-9382-0

642 Tian, L., & Shi, W. (2014). Soil peroxidase regulates organic matter decomposition through
643 improving the accessibility of reducing sugars and amino acids. *Biology and Fertility of*
644 *Soils, 50*, 785-794. https://doi.org/10.1007/s00374-014-0903-1
645 Tischer, A., Potthast, K., & Hamer, U. (2014). Land-use and soil depth affect resource and
646 microbial stoichiometry in a tropical mountain rainforest region of southern Ecuador.
647 *Oecologia, 175*, 375-393. https://doi.org/10.1007/s00442-014-2894-x
648 Tong, X., Brandt, M., Yue, Y., Horion, S., Wang, K., Keersmaecker, W. D., . . . Fensholt, R.
649 (2018). Increased vegetation growth and carbon stock in China karst via ecological
650 engineering. *Nature Sustainability, 1*, 44-50. https://doi.org/10.1038/s41893-017-0004-x
651 Trap, J., Riah, W., Akpa-Vinceslas, M., Bailleul, C., Laval, K., & Trinsoutrot-Gattin, I. (2012).
652 Improved effectiveness and efficiency in measuring soil enzymes as universal soil quality
653 indicators using microplate fluorimetry. *Soil Biology and Biochemistry, 45*, 98-101.
654 https://doi.org/https://doi.org/10.1016/j.soilbio.2011.10.010
655 Vitousek, P. M., Porder, S., Houlton, B. Z., & Chadwick, O. A. (2010). Terrestrial phosphorus
656 limitation: mechanisms, implications, and nitrogen-phosphorus interactions. *Ecological*
657 *Applications, 20*, 5-15. https://doi.org/10.1890/08-0127.1
658 Wang, S. J., Liu, Q. M., & Zhang, D. F. (2004). Karst rocky desertification in southwestern
659 China: Geomorphology, landuse, impact and rehabilitation. *Land Degradation &*
660 *Development, 15*, 115-121. https://doi.org/10.1002/ldr.592
661 Wang, R., Dungait J. A. J., Creamer C. A., Cai J., Xu Z., Zhang Y., Ma Y., Jiang Y. (2015).
662 Carbon and nitrogen dynamics in soil aggregates under long-term N and water addition in
663 a temperate steppe grassland. *Soil Science Society of America Journal 79*, 527-535.
664 https://doi.org/10.2136/sssaj2014.09.0351
665 Waring, B. G., Weintraub, S. R., & Sinsabaugh, R. L. (2014). Ecoenzymatic stoichiometry of
666 microbial nutrient acquisition in tropical soils. *Biogeochemistry, 117*, 101-113.
667 https://doi.org/10.1007/s10533-013-9849-x
668 Wen, L., Li, D., Yang, L., Luo, P., Chen, H., Xiao, K., . . . Wang, K. (2016). Rapid recuperation
669 of soil nitrogen following agricultural abandonment in a karst area, southwest China.
670 *Biogeochemistry, 129*, 341-354. https://doi.org/10.1007/s10533-016-0235-3

671 Xu, J. T., Yin, R. S., Li, Z., & Liu, C. (2006). China's ecological rehabilitation: Unprecedented
672 efforts, dramatic impacts, and requisite policies. *Ecological Economics*, *57*, 595-607.
673 https://doi.org/10.1016/j.ecolecon.2005.05.008
674 Xu, Z. W., Yu, G. R., Zhang, X. Y., Ge, J. P., He, N. P., Wang, Q. F., & Wang, D. (2015). The
675 variations in soil microbial communities, enzyme activities and their relationships with
676 soil organic matter decomposition along the northern slope of Changbai Mountain.
677 *Applied Soil Ecology*, *86*, 19-29. https://doi.org/10.1016/j.apsoil.2014.09.015
678 Xu, Z. W., Yu, G. R., Zhang, X. Y., He, N. P., Wang, Q. F., Wang, S. Z., . . . Wang, C. Y.
679 (2017). Soil enzyme activity and stoichiometry in forest ecosystems along the North-South
680 Transect in eastern China (NSTEC). *Soil Biology & Biochemistry*, *104*, 152-163.
681 https://doi.org/10.1016/j.soilbio.2016.10.020
682 Yang, Z., Yu, T., Hou, Q., Xia, X., Feng, H., Huang, C., . . . Zhang, M. (2014). Geochemical
683 evaluation of land quality in China and its applications. *Journal of Geochemical*
684 *Exploration*, *139*, 122-135. https://doi.org/10.1016/j.gexplo.2013.07.014
685 Young, I. M., & Crawford, J. W. (2004). Interactions and self-organization in the soil-microbe
686 complex. *Science*, *304*, 1634-1637. https://doi.org/10.1126/science.1097394
687 Zhang, W., Zhao, J., Pan, F. J., Li, D. J., Chen, H. S., & Wang, K. L. (2015a). Changes in
688 nitrogen and phosphorus limitation during secondary succession in a karst region in
689 southwest China. *Plant and Soil*, *391*, 77-91. https://doi.org/10.1007/s11104-015-2406-8
690 Zhang, X. Y., Dong, W. Y., Dai, X. Q., Schaeffer, S., Yang, F. T., Radosevich, M., . . . Sun, X.
691 M. (2015b). Responses of absolute and specific soil enzyme activities to long term
692 additions of organic and mineral fertilizer. *Science of the Total Environment*, *536*, 59-67.
693 https://doi.org/10.1016/j.scitotenv.2015.07.043
694 Zhang, X. Y., Yang, Y., Zhang, C., Niu, S. L., Yang, H., Yu G. R., . . . Sun, X. M. (2018).
695 Contrasting responses of phosphatase kinetic parameters to nitrogen and phosphorus
696 additions in forest soils. *Functional Ecology*, 32: 106–116. https://doi.org/10.1111/1365-
697 2435.12936
698 Zhang, Z. S., Dong, X. J., Liu, Y. B., Li, X. R., Jia, R. L., Hu, Y. G., . . . Huang, L. (2012). Soil
699 oxidases recovered faster than hydrolases in a 50-year chronosequence of desert

revegetation. *Plant and Soil, 358*, 275-287. http://doi.org/10.1007/s11104-012-1162-2

Zhu, H. H., He, X. Y., Wang, K. L., Su, Y. R., & Wu, J. S. (2012). Interactions of vegetation succession, soil bio-chemical properties and microbial communities in a Karst ecosystem. *European Journal of Soil Biology, 51*, 1-7. https://doi.org/10.1016/j.ejsobi.2012.03.003

Tables

Table 1 Characteristics of the sampled sloping cropland, abandoned cropland, shrubland, secondary forest, and primary forest.

Land use type	Dominant species	Slope (°)	Altitude (m)	Disturbance history
Sloping cropland	*Zea mays*	5-10	1421-1435	Maize rotated with soybeans and rape seed oil. Animal or human excreta combined with urea or compound fertilizer applied at regular intervals.
Abandoned cropland	*Conyza canadensis, Artemisia dubia,*	12-31	1405-1431	Abandoned for ~2-3 years.
Shrubland	*Rubusparvifoliu sp., Rubusinopertus sp., Litsearubescen sp., Rosacymosa sp., Artemisia sp., Rhus chinensis*	38-49	1447-1484	Recovering naturally for ~2-3 years.
Secondary forest	*Rhus chinensis, Litsea rubescens Lecomte., Populus adenopoda Maxim., Toona sinensis* (*A.Juss.*) *Roem*.	40-46	1449-1471	Recovering naturally for ~ 10 years
Primary forest	*Itea yunnanensis, Carpinus pubescens, Lithocarpus confinis*	33-40	1421-1503	More than 100 years without disturbance.

Table 2 Mean (n= 4, ± 1 standard deviation) soil properties at each depth for each land use. Definition of abbreviations: SWC: soil water content, SOC: soil organic carbon, DOC: dissolved organic carbon, TN: total nitrogen, AN: available nitrogen, TP: total phosphorus, available P: available phosphorus.

Soil properties	Depth (cm)	Sloping cropland	Abandoned cropland	Shrub land	Secondary forest	Primary forest
SWC (%)	0-10	36 ± 1^{B}	41 ± 2^{AB}	46 ± 9^{AB}	63 ± 3^{Aa}	61 ± 3^{Aa}
	10-30	34 ± 2^{B}	38 ± 2^{A}	41 ± 1^{AB}	50 ± 4^{Aab}	45 ± 3^{ABb}
	30-50	34 ± 2	39 ± 2	38 ± 3	40 ± 2^{bc}	39 ± 2^{b}
	>50	35 ± 1	-	-	34 ± 2^{c}	38 ± 3^{b}
pH	0-10	6.8 ± 0.5	7.2 ± 0.2	6.9 ± 0.1	7.0 ± 0.3	6.6 ± 0.5
	10-30	6.9 ± 0.2	7.3 ± 0.2	6.7 ± 0.2	7.0 ± 0.2	7.1 ± 0.3
	30-50	7.2 ± 0.3	7.4 ± 0.2	7.1 ± 0.1	7.2 ± 0.1	7.2 ± 0.3
	>50	7.1 ± 0.1	-	-	7.7 ± 0.2	7.1 ± 0.3
SOC (g kg^{-1})	0-10	24.7 ± 1.45^{Da}	30.3 ± 2.67^{D}	46.7 ± 5.16^{Ca}	64.7 ± 0.61^{Ba}	77.9 ± 5.61^{Aa}
	10-30	21.0 ± 0.84^{Ba}	27.6 ± 4.27^{B}	35.7 ± 1.84^{ABa}	51.3 ± 6.43^{Aa}	47.6 ± 9.40^{Ab}
	30-50	11.9 ± 2.98^{Bb}	24.1 ± 4.93^{AB}	20.8 ± 2.37^{ABb}	27.6 ± 4.53^{ABb}	35.7 ± 2.97^{Abc}
	>50	8.0 ± 1.64^{Bb}	-	-	15.4 ± 2.21^{ABb}	21.8 ± 3.35^{Ac}
DOC (mg kg^{-1})	0-10	29 ± 2.2^{Ba}	49 ± 9.0^{B}	138 ± 16.1^{A}	85 ± 21.6^{AB}	140 ± 27.4^{Aa}
	10-30	24 ± 2.0^{ab}	39 ± 7.6	80 ± 24.3	93 ± 26.3	73 ± 7.6^{ab}

	30-50	22 ± 3.1^{ab}	28 ± 2.8	59 ± 22.8	44 ± 8.3	68 ± 17.5^{ab}
	>50	17 ± 1.5^{b}	-	-	34 ±11.1	40±6.1^{b}
TN (g kg^{-1})	0-10	2.5 ± 0.22^{Ba}	3.1 ± 0.18^{B}	4.5 ± 0.47^{Ba}	4.5 ± 1.04^{Ba}	7.3 ± 0.33^{Aa}
	10-30	2.1 ± 0.09^{Bab}	2.7 ± 0.30^{B}	3.1 ± 0.08^{ABb}	4.4 ± 0.49^{Aa}	4.6 ± 0.48^{Aab}
	30-50	1.5 ± 0.23^{Bb}	2.4 ± 0.44^{AB}	2.1 ± 0.13^{Bb}	2.7 ± 0.41^{ABab}	3.7 ± 0.15^{Ab}
	>50	1.5 ± 0.39^{b}	-	-	1.8 ± 0.21^{b}	3.7 ± 1.11^{b}
AN (mg kg^{-1})	0-10	40 ± 6.1^{BC}	24 ± 3.7^{C}	48 ± 4.8^{AB}	57 ± 7.0^{ABa}	70 ± 3.0^{Aa}
	10-30	30 ± 5.1	23 ± 2.4	52 ± 13.1	37 ± 7.8^{ab}	31 ± 1.9^{b}
	30-50	25 ± 6.0^{B}	27 ± 3.8^{B}	49 ± 4.8^{A}	25 ± 1.8^{Bb}	23 ± 1.8^{Bbc}
	>50	22 ± 1.3	-	-	21 ± 5.1^{b}	20 ± 2.5^{c}
TP (mg kg^{-1})	0-10	487 ± 29^{Ba}	503 ± 42^{Ba}	348 ± 22^{Ba}	377 ± 26^{Ba}	1180 ± 173^{A}
	10-30	339 ± 39^{Bab}	404 ± 36^{Bab}	275 ± 4^{Bb}	323 ± 32^{Bab}	841 ± 66^{A}
	30-50	271 ± 46^{Bb}	332 ± 38^{Bb}	229 ± 11^{Bb}	222 ± 25^{Bb}	765 ± 93^{A}
	>50	276 ± 46^{Bb}	-	-	221 ± 14^{Bb}	643 ± 132^{A}
AP (mg kg^{-1})	0-10	4 ± 0.8^{A}	2 ± 0.4^{B}	4 ± 0.8^{AB}	4 ± 0.8^{AB}	9 ± 2.8^{A}
	10-30	3 ± 1.2^{B}	1 ± 0.3^{B}	4 ± 0.8^{B}	2 ± 0.8^{B}	12 ± 3.5^{A}
	30-50	3 ± 0.7^{A}	1±0.7^{B}	3 ± 0.5^{AB}	2 ± 0.6^{AB}	7 ± 2.4^{A}
	>50	3 ± 0.8^{B}	-	-	3 ± 0.5^{B}	12 ± 3.7^{A}

SOC:TP	0-10	50.8 ± 2.35^{Bab}	61.8 ± 8.24^{B}	137.6 ± 19.84^{Aa}	173.8 ± 11.22^{Aa}	72.0 ± 15.15^{Ba}
	10-30	64.3 ± 7.31^{Ba}	68.5 ± 9.40^{B}	130.3 ± 9.40^{Aab}	165.7 ± 29.95^{Aa}	56.7 ± 10.08^{Bab}
	30-50	42.6 ± 4.81^{Bbc}	70.3 ± 10.54^{B}	90.4 ± 7.59^{ABb}	124.6 ± 18.78^{Aab}	49.1 ± 7.66^{Bab}
	>50	29.5 ± 4.80^{Bc}	-	-	71.1±12.90^{Ab}	35.3 ± 3.47^{Bb}
TN:TP	0-10	5.2 ± 0.45^{B}	6.2 ± 0.64^{B}	12.9 ± 0.57^{A}	11.8±2.76^{AB}	6.7 ± 1.24^{AB}
	10-30	6.6 ± 0.80^{BC}	6.6 ± 0.63^{BC}	11.4 ± 0.33^{AB}	14.2±2.36^{A}	5.5 ± 0.46^{C}
	30-50	5.6 ± 0.49^{B}	7.2 ± 0.82^{AB}	9.0 ± 0.25^{AB}	12.6 ± 2.37^{A}	5.0 ± 0.39^{B}
	>50	5.3 ± 0.51	-	-	8.1 ± 1.26	5.6 ± 1.28

Superscripted uppercase letters indicate significant different sub-groups between land uses at $p < 0.05$ in each soil layer, while lowercase letters indicate significant different depth sub-groups in each land use.

Table 3 Pearson correlation analyses of the soil enzyme activities and soil properties.

	SWC	pH	SOC	DOC	TN	AN	TP	available P	SOC:TN	SOC:TP	TN:TP
BG	**0.705****	**-0.272***	**0.730****	**0.526****	**0.653****	**0.549****	**0.448****	**0.372****	**0.355****	**0.275***	0.122
NAG	**0.639****	-0.171	**0.723****	**0.555****	**0.632****	**0.592****	**0.569****	**0.391****	**0.340****	0.197	0.030
BX	**0.673****	-0.154	**0.688****	**0.456****	**0.638****	**0.525****	**0.509****	**0.318****	**0.320****	0.181	0.035
CBH	**0.551****	-0.217	**0.585****	**0.412****	**0.522****	**0.505****	**0.462****	**0.238***	**0.268***	0.099	-0.046
LAP	**0.501****	-0.122	**0.462****	**0.411****	**0.389****	**0.306****	**0.373****	0.221	**0.241***	0.095	-0.050
AP	**0.638****	**-0.234***	**0.635****	**0.570****	**0.556****	**0.549****	**0.444****	**0.317****	**0.300***	**0.250***	0.118
PPO	**0.486****	-0.103	**0.564****	**0.282***	**0.544****	**0.326****	**0.373****	**0.353****	**0.257***	0.219	0.129
POD	**0.470****	-0.224	**0.543****	**0.338****	**0.579****	**0.348****	**0.515****	0.204	0.159	0.082	0.009
InBG:ln(NAG+LAP)	**0.321****	**-0.289***	**0.388****	**0.276***	**0.358****	**0.252***	**0.239***	0.160	0.195	0.135	0.194
ln(NAG+LAP):InAP	0.195	0.202	0.189	0.072	0.146	0.015	0.203	-0.097	0.083	-0.108	-0.014
InBG:lnAP	**0.479****	-0.122	**0.553****	**0.339****	**0.490****	**0.282***	**0.412****	0.096	**0.268***	0.06	0.194

* and ** indicate the significant difference at $p < 0.05$ and $p < 0.01$. Definition of abbreviations: SWC: soil water content, SOC: soil organic carbon, DOC: dissolved organic carbon, TN: total nitrogen, AN: available nitrogen, TP: total phosphorus, available P: available phosphorus, BG: β-1,4-glucosidase, CBH: β-D-1,4-cellobiosidase, BX: β-1,4-xylosidase, NAG: β-1,4-N-acetyl-glucosaminidase, LAP: Leucine aminopeptidase, AP: alkaline phosphatase, PPO: Polyphenol oxidase, POD: Peroxidase.

Figure captions

Fig. 1 Soil enzyme activities in soil profile under different land uses. Uppercase letters above error bars indicate significant differences between land use sub-sets at $p < 0.05$ in each soil depth, and lowercase letters indicate significant different soil depth sub-sets in each land use. Additional notation as follows - BG: β-1,4-glucosidase, CBH: β-D-1,4-cellobiosidase, BX: β-1,4-xylosidase, NAG: β-1,4-N-acetyl-glucosaminidase, LAP: Leucine aminopeptidase, AP: alkaline phosphatase, PPO: Polyphenol oxidase, POD: Peroxidase.

Fig. 2 Comparison of soil enzyme activity ratios among the land use types. The lnBG:ln(NAG+LAP) indicates the ratios of natural logarithm of C (BG) to natural logarithm of N (NAG+LAP) nutrient acquiring hydrolase activities; The lnBG:lnAP indicates the ratios of natural logarithm of C (BG) to natural logarithm of P (AP) nutrient acquiring hydrolase activities; ln(NAG+LAP):lnAP indicates the ratios of natural logarithm of N (NAG+LAP) to natural logarithm of P (AP) nutrient acquiring hydrolase activities. Uppercase letters indicate significant different between land uses at $p < 0.05$.

Fig. 3 Comparison of soil enzyme activity ratios among the depths. Lowercase letters indicate significant different between soil depths at $p < 0.05$

Supplementary Tables

Table S1 Functions and substrates of the enzymes quantified in this study. EC: enzyme commission, MUB: 4-Methylumbelliferone.

Table S2 Two-way ANOVA analysis of the soil properties. *, ** and *** indicate the significant difference at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

Table S3 Comparison of soil properties among the land use types. Lowercase letters indicate significant different between land use types at $p < 0.05$.

Table S4 Comparison of soil properties among the soil depths. Lowercase letters indicate significant different between soil depths at $p < 0.05$.

Table S2 Two-way ANOVA of soil properties (variables) with soil depth (Depth) and Land Use Type as factors.

	Depth	Land use type	Depth ×Land use type
SWC	**15.67*** **	**9.64*** **	**3.08** **
pH	2.49	1.40	0.37
SOC	**55.32*** **	**33.50*** **	**4.46*** **
DOC	**10.76*** **	**12.47*** **	1.84
TN	**19.41*** **	**21.74*** **	**2.09***
AN	**17.22*** **	**9.45*** **	**3.79** **
TP	**13.14*** **	**54.36*** **	1.30
available P	0.75	**15.31*** **	0.60
SOC:TN	**3.66***	**2.62***	0.89
SOC:TP	**12.16*** **	**35.42*** **	1.95
TN: TP	2.03	**20.23*** **	1.34
BG	**41.57*** **	**11.33*** **	**2.67***
BX	**20.30*** **	**9.91*** **	**3.08** **
CBH	**25.90*** **	**5.85*** **	**2.25***
NAG	**48.14*** **	**25.16*** **	**8.69*** **
LAP	**6.17** **	**5.22** **	**2.2***
AP	**17.56*** **	**10.75*** **	**2.53***
PPO	**43.04*** **	**18.04*** **	**2.39***
POD	**38.20*** **	**12.82*** **	**2.84***
lnBG:ln(NAG+LAP)	**15.36*** **	**3.63***	1.64
ln(NAG+LAP):lnAP	1.50	**3.31***	0.67
lnBG:lnAP	**40.19*** **	**3.96** **	1.36

*, ** and *** indicate the significant difference at $p < 0.05$, $p < 0.01$ and $p < 0.001$. Definition of abbreviations: SWC: soil water content, SOC: soil organic carbon, DOC: dissolved organic carbon, TN: total nitrogen, AN: available nitrogen, TP: total phosphorus, available P: available phosphorus, BG: β-1,4-glucosidase, CBH: β-D-1,4-cellobiosidase, BX: β-1,4-xylosidase, NAG: β-1,4-N-acetyl-glucosaminidase, LAP: Leucine aminopeptidase, AP: alkaline phosphatase, PPO: Polyphenol oxidase, POD: Peroxidase.

Table S3 Comparison of soil properties among the land use types.

Soil properties	Slopping cropland	Abandoned cropland	Shrubland	Secondary forest	Primary forest
SWC (%)	35 ± 1^{B}	39 ± 1^{AB}	42 ± 3^{AB}	48 ± 3^{A}	46 ± 3^{A}
SOC (g kg^{-1})	16.4 ± 1.92^{C}	27.3 ± 2.26^{BC}	34.4 ± 3.67^{AB}	41.4 ± 5.4^{A}	45.8 ± 5.96^{A}
DOC (mg kg^{-1})	23 ± 1.5^{C}	39 ± 4.5^{C}	92 ± 15.0^{A}	66 ± 10.9^{B}	80 ± 12.1^{AB}
TN (g kg^{-1})	1.9 ± 0.16^{C}	2.7 ± 0.19^{BC}	3.2 ± 0.34^{B}	3.4 ± 0.42^{B}	4.8 ± 0.47^{A}
AN (mg kg^{-1})	29 ± 2.9^{B}	24 ± 1.8^{B}	50 ± 4.5^{A}	36 ± 4.6^{B}	36 ± 5.3^{B}
TP (mg kg^{-1})	343 ± 29^{BC}	413 ± 29^{B}	283 ± 16^{C}	291 ± 21^{C}	858 ± 75^{A}
available P (mg kg^{-1})	3 ± 0.4^{B}	2 ± 0.3^{B}	3 ± 0.4^{B}	3 ± 0.4^{B}	10 ± 1.5^{A}
SOC:TN	8.2 ± 0.53^{B}	9.9 ± 0.26^{AB}	10.8 ± 0.64^{AB}	13.1 ± 2.30^{A}	9.3 ± 0.55^{B}
SOC:TP	46.8 ± 3.99^{C}	66.9 ± 5.05^{B}	119.4 ± 9.27^{A}	138.0 ± 13.77^{A}	53.3 ± 5.64^{BC}
TN:TP	5.7 ± 0.29^{B}	6.7 ± 0.39^{B}	11.1 ± 0.54^{A}	11.9 ± 1.20^{A}	5.7 ± 0.45^{B}
BG (nmol h^{-1} g^{-1})	148 ± 35^{B}	110 ± 25^{B}	99 ± 18^{B}	271 ± 63^{AB}	393 ± 95^{A}
NAG (nmol h^{-1} g^{-1})	76 ± 22^{B}	32 ± 7^{B}	50 ± 8^{B}	201 ± 54^{AB}	303 ± 78^{A}
BX (nmol h^{-1} g^{-1})	24 ± 5^{B}	34 ± 6^{B}	32 ± 4^{B}	50 ± 9^{AB}	80 ± 18^{A}
CBH (nmol h^{-1} g^{-1})	30 ± 9^{AB}	23 ± 6^{AB}	12 ± 2^{B}	32 ± 8^{AB}	58 ± 16^{A}
LAP (nmol h^{-1} g^{-1})	188 ± 40^{B}	342 ± 58^{AB}	297 ± 57^{AB}	327 ± 54^{AB}	484 ± 84^{A}
AP (nmol h^{-1} g^{-1})	898 ± 135^{B}	474 ± 50^{B}	816 ± 62^{B}	1517 ± 239^{A}	1672 ± 311^{A}
PPO (μmol h^{-1} g^{-1})	26 ± 4^{AB}	24 ± 3^{AB}	20 ± 2^{B}	36 ± 4^{A}	34 ± 4^{A}
POD (μmol h^{-1} g^{-1})	44 ± 4^{AB}	32 ± 4^{B}	32 ± 2^{B}	46 ± 4^{AB}	55 ± 5^{A}
lnBG:ln(NAG+LAP)	0.84 ± 0.05^{AB}	0.76 ± 0.03^{B}	0.78 ± 0.03^{B}	0.86 ± 0.02^{A}	0.83 ± 0.02^{AB}
ln(NAG+LAP):lnAP	0.81 ± 0.04^{B}	0.95 ± 0.02^{A}	0.84 ± 0.03^{B}	0.84 ± 0.02^{B}	0.88 ± 0.02^{AB}
lnBG:lnAP	0.67 ± 0.03^{B}	0.72 ± 0.03^{AB}	0.65 ± 0.03^{B}	0.72 ± 0.02^{AB}	0.73 ± 0.03^{A}

Superscripted uppercase letters indicate significant different between land use types at $p < 0.05$.

Table S4 Comparison of soil properties among the soil depths.

Soil properties	0-10 cm	10-30 cm	30-50cm	> 50 cm
SWC (%)	49±3^{a}	42±2^{b}	38±1^{b}	36±1^{b}
SOC (g kg^{-1})	48.9±4.85^{a}	36.6±3.43^{a}	24±2.32^{b}	15.1±2.29^{b}
DOC (mg kg^{-1})	88±12.5^{a}	62±8.9^{b}	44±6.7^{bc}	30±4.6^{c}
TN (g kg^{-1})	4.4±0.44^{a}	3.4±0.26^{ab}	2.5±0.21^{b}	2.4±0.5^{b}
AN (mg kg^{-1})	48±4.1^{a}	34±3.7^{b}	30±2.7^{bc}	21±1.5^{c}
TP (mg kg^{-1})	579±78^{a}	436±50^{b}	364±51^{b}	395±76^{b}
SOC:TN	12.2±1.72^{a}	10.6±0.27^{a}	9.5±0.45^{ab}	7.1±0.86^{b}
SOC:TP	8.6±0.92^{a}	8.9±0.90^{a}	7.9±0.78^{ab}	6.2±0.66^{b}
BG (nmol h^{-1} g^{-1})	451±69^{a}	226±40^{b}	55±6^{c}	53±9^{c}
NAG (nmol h^{-1} g^{-1})	312±64^{a}	152±33^{b}	24±4^{c}	27±7^{c}
BX (nmol h^{-1} g^{-1})	82±15^{a}	39±5^{b}	23±3^{b}	30±7^{b}
CBH (nmol h^{-1} g^{-1})	71±13^{a}	22±4^{b}	13±2^{b}	16±3^{b}
LAP (nmol h^{-1} g^{-1})	468±70^{a}	349±37^{ab}	211±44^{b}	250±63^{b}
AP (nmol h^{-1} g^{-1})	1698±254^{a}	1227±200^{ab}	611±43^{b}	788±84^{b}
PPO (μmol h^{-1} g^{-1})	40±3^{a}	32±3^{a}	18±2^{b}	21±3^{b}
POD (μmol h^{-1} g^{-1})	58±4^{a}	46±3^{b}	26±2^{c}	40±3^{b}
lnBG:ln(NAG+LAP)	0.93±0.02^{a}	0.85±0.03^{b}	0.74±0.03^{c}	0.72±0.05^{c}
lnBG:lnAP	0.83±0.02^{a}	0.74±0.02^{b}	0.60±0.02^{c}	0.59±0.03^{c}

Superscripted lowercase letters indicate significant different between soil depths at $p < 0.05$.