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Lower Sensitivity of Soil Carbon and Nitrogen to Regional Temperature Change in Karst Forests Than in Non-Karst Forests

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Abstract: Lithology has such an important effect on the sustainability of soil carbon (C) pools. Forests are an important part of terrestrial C sinks; yet, it is unclear whether their soil carbon sensitivity to temperature changes is regulated by lithology, especially in karst ecosystems, which are widely distributed globally. Along a climate gradient in the subtropical region of southwest China, we compared the contents of soil organic C (SOC) and total N (TN) in karst and non-karst forests. The data were analyzed and processed using ANOVA, regression analysis, and random forest. The results showed that the karst forests had significantly higher SOC and TN contents but lower ratio of SOC to TN (C:N) than non-karst forests, mainly because of the higher soil calcium (Ca) content and microbial biomass. With rising mean annual temperature (MAT), SOC and TN contents in non-karst forests significantly decreased, whereas in karst forests they were not correlated with MAT; while, the opposite was true for C:N. In karst forests, soil Ca constrain warming induced decomposition of SOC and TN by forming stable complexes with SOM through exchangeable Ca, and by promoting aggregate stability through the role of calcium carbonate. The correlation between δ^{13}C and the logarithm of SOC concentration also supported that conclusion. In karst forests compared to non-karst forests, soil C pools are larger and less sensitive to regional temperature change. Nevertheless, climate warming may still accelerate soil C loss in karst forests by increasing microbial C limitation. Thus, soil C sequestration potential and loss risk coexist in karst areas. The ratio of SOC to TN (C:N) is regulated through appropriate management measures in the process of karst vegetation restoration, thus promoting long-term stable sequestration of soil carbon pools.

Keywords: climate warming; calcium; Karst ecosystems; lithology; soil organic matter

1. Introduction

The decomposition of soil organic carbon (SOC), which is the main source of CO_2 in the atmosphere, is the greatest carbon reservoir in terrestrial ecosystems, holding about three times as much carbon (C) as plants and twice as much as the atmosphere [1–4]. In part because of their sequestration of C and therefore their ability to mitigate climate change, forest ecosystems have been receiving increasing attention [5,6]. For example, Bastin et al. (2019) predicted that worldwide tree restoration might result in a 25% reduction in the atmospheric C pool [7]. The C stock of forest soils, however, varies greatly depending on soil type, climate, soil depth, tree species, and geological background [8–11].

SOC sequestration illustrates the equilibrium between C inputs and outputs [12]. The key controllers of these inputs and outputs are the environment, soil microbial decom-

posers, substrate quantity, and substrate quality [13–15]. Additionally, C output is closely related to a soil's ability to stabilize SOC, which depends in part on the texture, minerals, and aggregate composition of the soil [16]. SOC content also depends on soil total nitrogen (TN) content, i.e., long-term SOC sequestration is limited by TN content [17,18] as well as by the soil C:N ratio [19]. Additionally, temperature, precipitation, and other climatic factors may affect these factors simultaneously. Climate factors can have intricate implications for SOC. Forest SOC stocks in have been found to increase [20], decrease [21,22], or change negligibly [23] as mean annual temperature (MAT) increases. Given global climate change [24], forest productivity and the behavior of soil organisms will be influenced by temperature and precipitation [6,25,26]. Predicting the soil's capacity to store carbon under potential future climate change scenarios will require a thorough understanding of the relationship between climate conditions and SOC and TN concentration in forest ecosystems.

Globally, about 15% of the land area is occupied by karst ecosystems formed on carbonate rocks (such as limestone and dolomite) [27]. The specificity of these ecosystems is demonstrated by the high content of Ca and magnesium (Mg) in soil, shallow soil depth, high pH, and the presence of exposed carbonate bedrock [28]. The unique geological background and hydrologic processes of karst regions create fragile ecosystems that are highly sensitive to disturbance, especially to human disturbance [29]. Karst regions have previously been studied mainly at the ecosystem level or within a small region, i.e., regional-scale studies have not been conducted [30,31]. Climate change affects SOC and TN in karst forests, which is important for understanding global C budget.

In addition, karst and non-karst soils in the same region have been compared in limited previous studies about land use and soil properties [15,32]. Earth's surface is defined by the lithology of its parent materials, namely their mineralogy, geochemistry, and physical properties [33]. Different effects of lithology have been recorded on soil characteristics, soil microorganisms, and plant function and composition [34,35]. Studies on the impact of lithology on soil C and TN and its influencing variables, however, are still underreported. Comparing the responses of SOC and TN to climate gradients under different lithologies contributes to the assessment of the specificity of SOC dynamics and prediction of the magnitude and direction of its response to climate change in karst regions. Southwest China, one of the three largest karst areas within the world [27], is experiencing more frequent extreme climate events than in the past [36]. Widely distributed karst soils (calcareous soils) are interwoven with non-karst soils (mainly ferralsol). The reactions of SOC and TN to climate change in karst and non-karst forests can thus be compared in southwest China.

In the current study, we evaluated the SOC and TN contents and their regulating elements in karst and non-karst forests under various climatic gradients in the karst region of southwest China. Because the high Ca and Mg contents in karst soils can help stabilize soil organic matter (SOM) [37,38], and the formation of Ca-rich carbonate rocks in karst areas, calcareous soils promote OM-Ca^{2+} binding, which in turn maintains high SOC and TN accumulation. The regulation of SOC and TN accumulation by Ca^{2+} is mainly due to the OM-Ca^{2+} mineral complex. Therefore, we hypothesize that karst forests have higher SOC and TN contents, and they are less sensitive to climate warming compared to non-karst forests.

2. Methods and Materials

2.1. Study Area

The study region was situated in the southwest Chinese provinces of Guizhou and Guangxi (between 22°34′ and 27°55′ N, 104°78′ and 108°65′ E). The study area is between 146 and 2890 m above sea level (from south to north). With a typical subtropical monsoon climate, its mean annual temperature (MAT) ranges from 12.6 °C to 21.8 °C and its mean annual precipitation (MAP) from 1013 mm to 1607 mm. Meteorological data (including temperature and rainfall) for 2000–2015 were obtained from the National Meteorological Information Center of China (http://data.cma.cn/, accessed on 20 June 2018). A karst

region's lithology is dominated by limestone, dolomite, and their mixtures, while non-karst regions' are clastic.

2.2. Experimental Design and Sample Collection

The sample site survey and soil sampling were conducted from August to October 2018. Soil samples were collected in 7 counties, namely Jinsha, Duyun, and Shuicheng in Guizhou Province Du'an, Huanjiang, Longzhou, and Masan in Guangxi Province (Figure 1). Secondary forests with an estimated age of 60 ± 5 years were selected in both karst and non-karst regions in each county. The local forestry management provided historical and relevant information about the selected forest sites (Table S1). The soil type and geochemical background of the sampling sites must match those of the other sites chosen; the slope should typically be between 15° and 20°, and the sites should face south or southeast. The lithology of the study sites was confirmed by bedrock sample collection and experimental analysis. In total, 42 sites were sampled, with two lithology type and three replicates in each county.
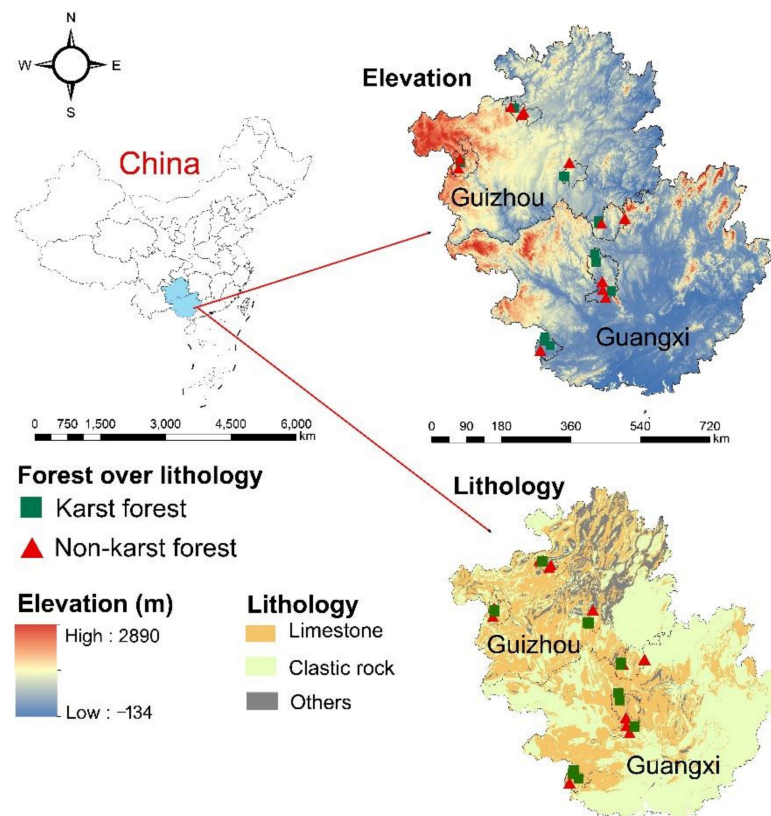

Figure 1. Map showing the study's sampled karst and non-karst forests. A total of 42 sites (21 for each sampling site) were sampled.

A 30×30 m sample plot was established at each site and mineral soil samples were collected at a depth of 15 cm in each plot (after removal dead leaves from the surface if present). Twenty soil cores were collected randomly at each site using a soil auger (38 mm diameter) and mixed thoroughly. The soil was strained through a 2 mm mesh sieve and visible roots and stones were removed with a tweezer. Phospholipid fatty acid (PLFA) analysis was performed using fresh soil stored at -20 °C. The other part of air-dried soil was used to determine soil physicochemical properties.

2.3. Sample Processing and Analysis

The pH of the soil was measured with a pH meter (FE20K; Mettler-Toledo, Greifensee, Switzerland) after mixing at a soil to water ratio of 1:2.5. pH was then converted to H^+

concentration and statistically analyzed, and then back-calculated to pH. SOC content was determined by concentrated sulfuric acid–potassium dichromate oxidation with ferrous sulfate titration. After carbonate removal by H_3PO_4, the C isotope ratios of SOC (δ^{13}C-SOC, in ‰ of PDB) were measured using a MAT 253 isotope ratio mass spectrometer (Thermo Finnigan MAT, Bremen, Germany). An elemental analyzer was used to measure the soil TN (vario MAX; Elementar, Langenselnold, Hesse, Germany). ICP-OES was used to determine the amount of soil-exchangeable calcium (Ca^{2+}) and magnesium (Mg^{2+}) using the ammonium acetate exchange method. (Agilent. Santa Clara, CA, USA). The citrate–bicarbonate–dithionite and acid oxalate extraction methods were used to determine soil free iron oxide (Fe_o) and amorphous iron oxide (Fe_d). The content of $CaCO_3$ in soils was determined through acid–base titration [39]. The total Ca, Mg, and Fe concentrations of the soils were analyzed with an X-ray fluorescence spectrometer (Axios mAX; Malvern Panalytical B.V., Almelo.The Netherlands), and the final values (%) were represented as CaO, MgO, and Fe_2O_3, respectively.

Soil microbial biomass (MB) was assessed via PLFA analysis, which is considered an accurate measure of soil MB [40]. According to Bossio and Scow (1998), PLFA was extracted from 8 g of fresh soil and analyzed [41]. The abundance of bacterial PLFAs was calculated as the sum of i14:0,a15:0,i15:0,i16:0,16:1ω7c,17:0,a17:0,i17:0,cy17:0,18:0,18:1ω7c, and cy19:0 [42]. The abundance of fungal PLFAs was calculated as the sum of 18:1ω9c and 18:2ω6,9c [43]. The abundance of bacterial and fungal PLFAs was expressed as nmol g^{-1} dry soil.

2.4. Statistical Analysis

Before analysis, the data were checked for normal distribution and variance homogeneity. Differences in SOC, TN, and the ratio of SOC to TN (C:N), and the relationship between δ^{13}C-SOC content and the logarithm of SOC content between karst and non-karst forests were analyzed using one-way ANOVA and regression analysis. Redundancy analysis (RDA) was performed using Canoco v.5.0 (Centre for Biometry, Wageningen, The Netherlands) to elucidate the relationship of SOC and TN with soil properties. The significance of the RDA results based on 999 randomly generated data was evaluated using the Monte Carlo test. Unless otherwise specified, results were considered statistically significant at $p < 0.05$. The significance of the effect of each explanatory variable on SOC and TN was estimated using the "randomForest" "rfPermute" package in R 4.2.2 and the relative relevance of the explanatory variables was ranked using the percentage increase in mean squared error (%IncMSE). Data were statistically analyzed and plotted using OriginPro 2022 (OriginLab, Hampton, MA, USA) and SPSS 26.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Differences in Soil Properties between Karst and Non-Karst Forests

As shown in Table 1, soil pH was significantly lower in non-karst forests than in karst ($p < 0.05$). On average, the exchangeable Ca^{2+}, Mg^{2+}, and soil MB contents were about 4.3, 3.3, and 2.5 times lower in non-karst forests than in karst forests, respectively. Generally, $CaCO_3$, CaO, MgO, Fe_2O_3, and CaO/MgO were significantly lower in non-karst forests than in karst forests ($p < 0.05$), whereas Fe_d and field capacity were not significantly different.

Table 1. Soil properties of forests under different lithologies.

Soil Property	Karst Forest	Non-Karst Forest
Field capacity/%	48.27 (2.46)	49.71 (3.44)
Total porosity/%	62.67 (1.46)	61.98 (2.27)
pH	5.89 (a)	4.22 (b)
Ca/nmol·kg^{-1}	35.65 (3.05) a	6.75 (2.18) b
Mg/nmol·kg^{-1}	9.39 (1.78) a	2.20 (0.41) b
Fe$_d$/nmol·kg^{-1}	3.11 (0.33)	6.82 (1.12)
Fe$_o$/nmol·kg^{-1}	55.42 (4.71)	45.82 (4.05) b
CaCO$_3$/%	2.00 (0.21) a	0.92 (0.15) b
CaO/%	1.87 (0.51) a	0.36 (0.08) b
MgO/%	1.74 (0.23) a	0.80 (0.12) b
Fe$_2$O$_3$/%	9.43 (0.61) a	7.10 (0.82) b
CaO/MgO	1.11 (0.17) a	0.52 (0.12) b
MB/nmol·kg^{-1}	95.96 (8.91) a	38.40 (4.02) b

Ca, soil exchangeable Ca^{2+}; Mg, soil exchangeable Mg^{2+}; Fe$_d$, free iron oxide; Fe$_o$, amorphous iron oxide; MAP, mean annual precipitation; MAT, mean annual temperature; CaCO$_3$, calcium carbonate; CaO, calcium oxide; MgO, magnesium oxide; Fe$_2$O$_3$, ferric oxide; CaO/MgO, the ratio of CaO to MgO; MB, microbial biomass. At $p < 0.05$, different letters indicate a significant distinction between karst and non-karst sites. The data are shown as means and standard errors.

3.2. Contents of SOC and TN in Karst and Non-Karst Forests

As shown in Figure 2, the SOC and TN contents of non-karst forests were significantly lower than those of karst forests, whereas the opposite was true for soil C:N ($p < 0.05$). Furthermore, SOC, TN contents and C:N were more closely related to MAT than to MAP (Figure 3). In non-karst forests, SOC and TN content decreased significantly as MAT increased ($p < 0.05$), but was unrelated to MAT in karst soils (Figure 3b,d). However, C:N in karst soils decreased significantly with increasing MAT ($p < 0.05$, Figure 3f). In contrast to karst forests, δ^{13}C-SOC in non-karst forests decreased with increasing log-transformed SOC content ($p < 0.05$, Figure 4).
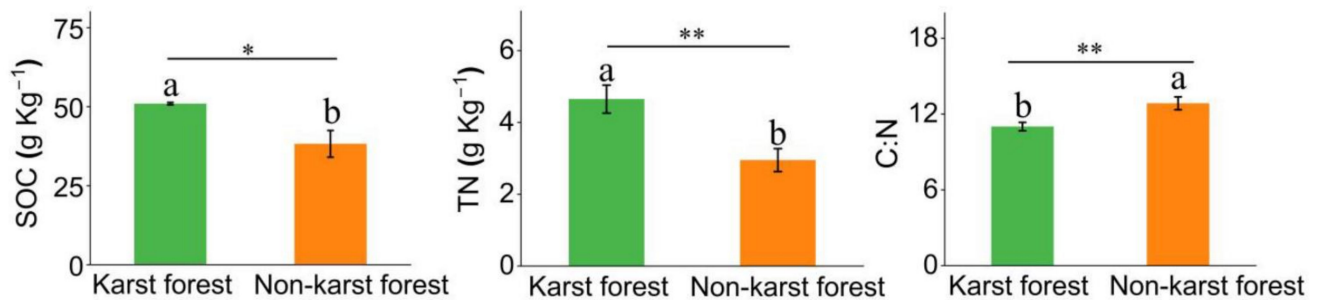

Figure 2. Forest soil organic carbon (SOC), total nitrogen (TN), and the ratio of SOC to TN (C:N) under different lithologies. At $p < 0.05$, different letters indicate a significant distinction between karst and non-karst sites. The data are shown as means and standard errors. * represents $p < 0.05$. ** represents $p < 0.01$.
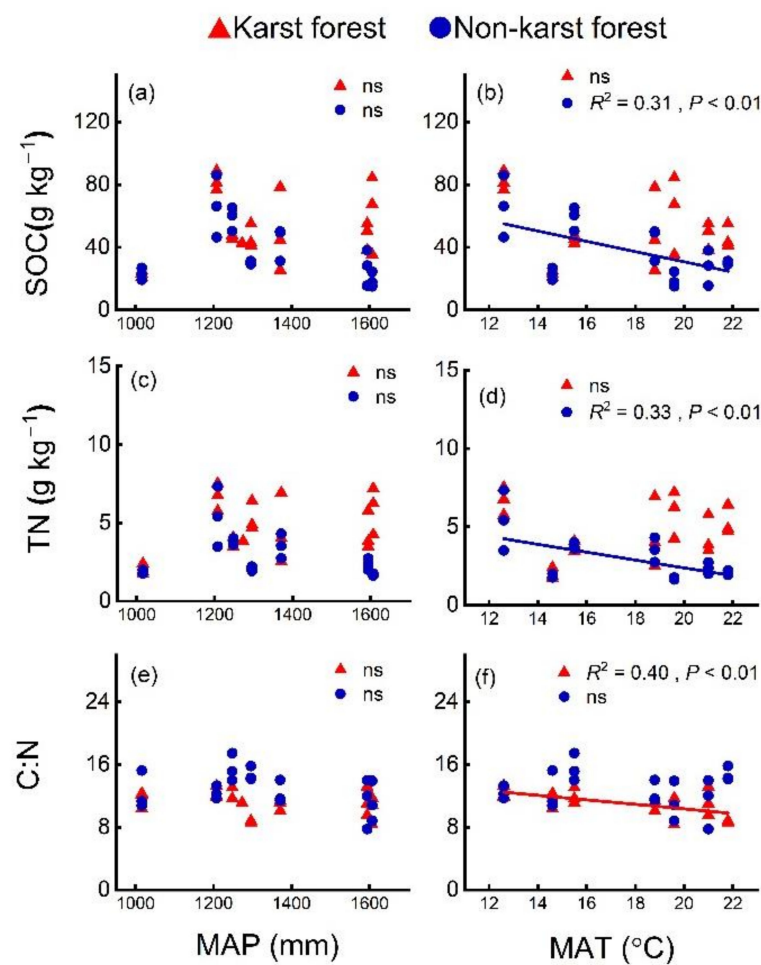
Figure 3. Relationships between mean annual precipitation (MAP) and temperature (MAT) and soil organic carbon (SOC), total nitrogen (TN), and the ratio of SOC to TN (C:N) in karst and non-karst forests. Relationship between SOC and MAP and MAT in karst and non-karst forests ((**a**,**b**), respectively), TN and MAP and MAT ((**c**,**d**), respectively), C:N and MAP and MAT ((**e**,**f**), respectively). ns, not significant.
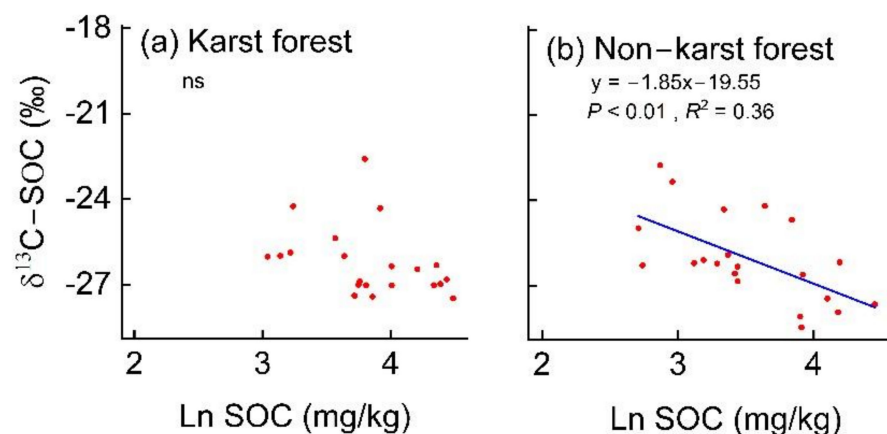

Figure 4. Relationship between the soil organic carbon (SOC) content in karst and non-karst forests and the logarithm of that content (δ^{13}C-SOC). ns, not significant. (**a**) Karst forest. (**b**) Non-karst forest.

3.3. Soil Properties Correlated with SOC and TN Contents

RDA results revealed that the relationships between SOC and TN contents and the measured soil properties differed between karst and non-karst soils (Figure 5). In karst

forests, SOC and TN were positively correlated with Ca^{2+}, $CaCO_3$, CaO/MgO, microbial biomass, total porosity (TP), field capacity (FC), and pH ($p < 0.05$). In non-karst forests, SOC and TN were positively correlated with Fe_o, TP, and FC, and negatively correlated with MAT ($p < 0.05$). Random forest results further revealed that Ca-related parameters (e.g., Ca^{2+}, CaO, $CaCO_3$, and CaO/MgO) and microbial biomass were the most important factors controlling SOC and TN variation in karst forests. In contrast, SOC and TN changes in non-karst forests are mainly controlled by TP, FC, pH, and Fe_o factors (Figure 6).
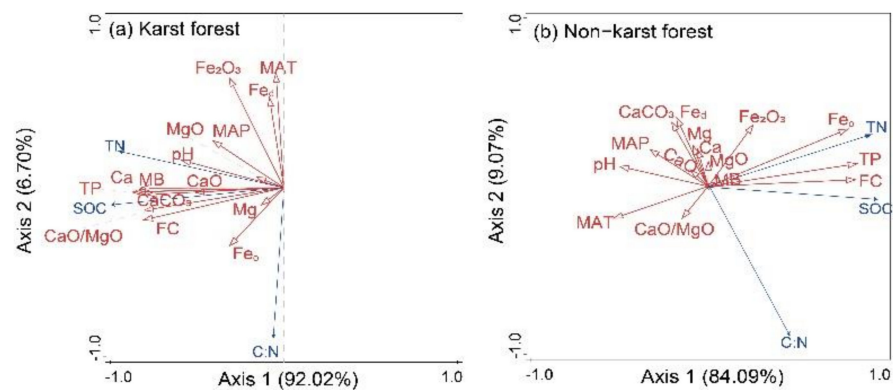

Figure 5. Effects of soil physicochemical properties on soil organic carbon (SOC), total nitrogen (TN), and the ratio of SOC to TN (C:N) in karst and non-karst forests. SOC, TN, and C:N are represented by blue arrows; soil physicochemical properties are represented by red arrows. Ca, soil exchangeable Ca^{2+}; Fe_o, amorphous iron oxide; Mg, soil exchangeable Mg^{2+}; Fe_d, free iron oxide; MAP, mean annual precipitation; FC, field capacity; TP, total porosity; MB, microbial biomass; $CaCO_3$, calcium carbonate; CaO, calcium oxide; CaO/MgO, the ratio of CaO to MgO; MgO, magnesium oxide; Fe_2O_3, ferric oxide; MAT, mean annual temperature. (**a**) Karst forest. (**b**) Non-karst forest.
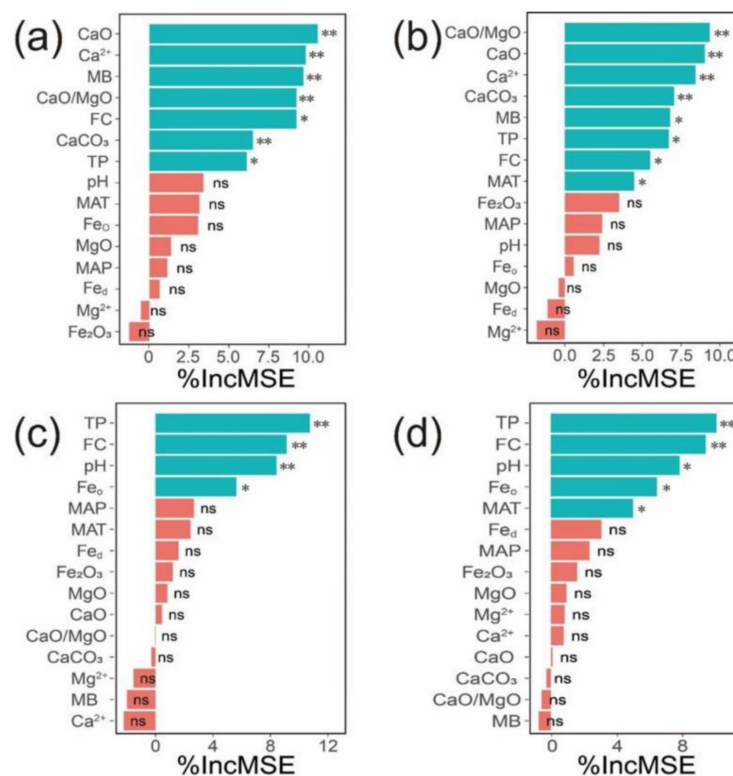

Figure 6. Relative importance of soil physicochemical properties in the variation of soil organic carbon and total nitrogen in karst ((**a**,**b**), respectively), and non-karst forests ((**c**,**d**), respectively). Importance was used to determine the percentage increase in mean square error of the random forest model. Ca, soil

exchangeable Ca^{2+}; Fe_o, amorphous iron oxide; Mg, soil exchangeable Mg^{2+}; Fe_d, free iron oxide; MAP, mean annual precipitation; FC, field capacity; TP, total porosity; MB, microbial biomass; $CaCO_3$, calcium carbonate; CaO, calcium oxide; CaO/MgO, the ratio of CaO to MgO; MgO, magnesium oxide; Fe_2O_3, ferric oxide; MAT, mean annual temperature. * represents $p < 0.05$. ** represents $p < 0.01$. ns, not significant.

4. Discussion

4.1. Differences in Soil Carbon and Nitrogen between Karst and Non-Karst Forests

Karst forests had significantly higher SOC and TN contents than non-karst forests (Figure 2), which is consistent with our hypothesis. SOC and TN contents are closely related to the lithology type of karst areas [12,43,44]. Lithology greatly affects soil pH, exchangeable cations, texture, and other soil properties [45], which may in turn affect SOM stability. Doetterl et al. (2015) [46], based on studies covering a wide range of climatic and geochemical conditions, showed that geochemistry is the main factor controlling soil C storage in Chile and the Antarctic Peninsula. It has been reported that SOC stocks differ significantly in some areas with different lithological characteristics, including areas with soils underlain by schist and granite and volcanic lava and schist-marble [47]. In contrast, another study found that calcareous and metamorphic soils differed in some soil properties (pH, texture, and exchange complex and free oxides) but not in SOC content [48]. These inconsistent results emphasize the importance of taking lithology into account when assessing soil C pools in a region underlain by different lithologies. In the present study, karst forests' SOC and TN contents were mainly due to the increased SOM stability of calcareous soils. In contrast, the changes in SOC and TN contents in non-karst forest soils were associated with TP, FC, pH, and Fe oxides (Figures 5 and 6). Due to the Ca-rich nature of carbonate rocks in karst areas, the calcareous soils they form are conducive to OM-Ca^{2+} binding, which in turn maintains a high accumulation of SOC and TN [28,49]. The regulation of SOC and TN accumulation by Ca^{2+} is mainly due to the formation of OM-Ca^{2+} mineral complexes that contribute to the stabilization of SOM [50]. In forest soils over clastic rock, amorphous Fe-alumina oxides are significantly enriched in the deeper layers of acidic soils, and its large specific surface area and denser hydroxyl sites contribute to the stabilization of SOC [51]. In addition, Fe_o can reduce the decomposition of microbial necrotic material, inseparable from the stability of soil aggregates [52]. Therefore, higher Fe_o content promotes C accumulation in non-karst forest soils. However, Fe_o content did not significantly differ between karst and non-karst, and Ca^{2+} contents in karst forest were significantly higher than in non-karst. Therefore, the SOC and TN contents of karst forests were much higher than those of non-karst (Table 1).

Differences in soil microbial abundance under different lithologies affected their SOC and TN contents. Microbial biomass was higher in karst forests than in non-karst forests (Table 1), suggesting that there are more organic matter decomposers in karst forests, but also more C and N fractions derived from microbial necromass [53]. As major components of SOM, SOC and TN are greatly affected by SOM transformation processes, which are controlled by soil microorganisms [54]. The differences in the relationship between SOC and TN with microbial biomass in karst and non-karst forests (Figures 5 and 6) may be due to the differences in pH and Ca (including CaO, $CaCO_3$, and Ca^{2+}) content [55]. Soil pH was much more acidic in non-karst forests than in karst forests (Table 1). The low pH in the non-karst soil is likely to restrict the growth of bacteria and fungi [56,57]. In addition, higher Ca content in karst forest soils improves soil structure and aeration, thus creating environments conducive to microbial growth [58]. Thus, karst forests have much higher microbial biomass than non-karst forests, which contributes to SOM buildup via microbial anabolism.

While the SOC and TN contents were greater in karst than in non-karst forests, the opposite was true for C:N. Chen et al. (2012) [32] found that the increment of TN content in the karst area was higher than that of SOC compared to the non-karst area, which is similar to our result. The main reason is that karst forests in subtropical climate zones tend

to be saturated with N, whereas non-karst forests are N-limited [59]. In general, global forests are N-limited, while tropical/subtropical regions are relatively N-rich [60]. However, Wen et al. (2016) found very high soil NO_3^- concentrations (66.5–120.9 mg N kg^{-1}) in karst forests, implying that karst areas have the potential for high NO_3^- leaching, saturating the microbial demand for N in karst soils [31]. Additionally, soil C:N is often used as an indicator of the decomposition or accumulation of SOM, and a lower ratio means that SOM is likely to decompose more rapidly [61]. Consequently, the relatively huge C pool in karst soils is "stable" only in the absence of soil disturbance. Therefore, ecological restoration measures for karst areas are particularly important.

4.2. Lower Temperature Sensitivity of SOC and TN in Karst Forests

SOC and TN contents were more consistently related to MAT than to MAP in the present study (Figure 3). Whether variability in SOC and TN contents is more closely related to MAT or to MAP has differed among previous studies [11,62,63]. The inconsistency suggests that the relationship between SOC and TN contents and climatic factors depends on the region. Given that all of the research sites experience significant precipitation, precipitation may not be the primary factor limiting plant growth or soil microbial activity in the current study [64]. In contrast, temperature greatly varied between study locations as a result of the vast altitude variation. Therefore, based on its effects on plant development and soil microbial activity, we can deduce that temperature was a major influence impacting SOM.

The correlations of SOC and TN contents with MAT differed between karst and non-karst forests, implying that lithology controls the temperature sensitivity of SOC and TN in forests. This is consistent with our hypothesis that SOC and TN in karst forests are less sensitive to warming than in non-karst forests. SOC and TN contents decreased in non-karst forests as MAT increased, which is consistent with the meta-analysis of global data [22]. Over long periods, higher temperatures are expected to promote soil microbial activity and to stimulate SOC decomposition [65]. Additionally, warmer regions usually accumulate more labile carbon than cooler regions. For example, Gutiérrez-Girón et al. (2015) [66] discovered that rising MAT led to an increase in labile SOC, such as dissolved organic C and microbial biomass. Thus, higher microbial decomposition rates and higher liable C contents in warmer regions may promote soil C decomposition such that less SOC and TN are retained in soils. Given that SOC and TN contents in non-karst soils were negatively correlated with MAT, the non-karst areas in the subtropical regions of southwest China could be highly sensitive to the global warming. In karst forests, in contrast, SOC and TN contents were not correlated with MAT. This result may be attributed to the unique characteristics of calcareous soils. As mentioned earlier, in Ca-rich soils, SOM stability is determined by soil exchangeable Ca^{2+} [61]. Stability of SOC and TN increases with strong interaction between exchangeable Ca^{2+} and SOM in the soil. For example, due to the high Ca content in karst soils, 90% of the SOC in calcareous soils exists in non-labile complexes of minerals and organic matter [38]. By definition, the residence time is longer for stabilized C than for labile C [67]. As a consequence, SOM in karst forests, which are rich in Ca^{2+}, $CaCO_3$, and CaO can be integrated into OM-Ca^{2+} complexes that are chemically protected from decomposition. In addition, $CaCO_3$ forms calcite crystals during the dissolution–precipitation process, which fills the porous structure of aggregates and enhances the stability of soil aggregates and occluded SOC [68]. Furthermore, the high levels of fungal PLFAs in karst forest soils may also benefit SOC sequestration. Fungal hyphae can enhance the formation of soil macroaggregates that help to physically protect organic C in the interior of soil aggregates [69].

The fact that karst forest soils help to stabilize SOC is also indicated by the linear relationship between δ^{13}C-SOC and the log-transformed SOC (log SOC) content (Figure 4). Previous studies have used the slope of the regression β between the δ^{13}C value and the logarithm of the SOC content as a proxy for SOC turnover. For example, higher SOC turnover rates were frequently related to more negative values (i.e., steeper slopes) [70–72].

In the present study, β values (−1.85) in non-karst forest soils were consistent with those in other studies (−2.67) [71,72]. In contrast, no relation between $\delta^{13}C$ and log SOC was found in karst forest (Figure 6a), perhaps because the majority of the SOC in karst forests remained undecomposed by soil microorganisms. Overall, our results suggest that SOC and TN in karst forests are more resistant to temperature changes.

In contrast to non-karst forests, soil C:N decreased with increasing MAT in karst forests (Figure 3). This may be mainly due to the simultaneous decrease in SOC and TN contents with increasing MAT in non-karst forests, resulting in no significant change in C:N ratios. Although SOC and TN did not decrease significantly with increasing MAT in karst forests, SOC showed a weak decreasing trend in higher temperature regions compared to TN, which led to a decrease in C:N with increasing MAT. It has been found that the N concentration in limestone ranged from 0.02 to 0.30 g kg^{-1} [73], and weathering of N-rich bedrock could increase the availability of soil N [74]. Therefore, the reason why N in karst forests does not decrease with increasing temperature may be due to the high weathering rate of karst bedrock in high temperature areas, which supplements the source of N input.

Although SOC and TN in karst forests are more resistant to temperature changes than those in non-karst forests, the decrease in C:N with increasing MAT suggests that microbial C limitation is intensifying in the context of global warming, leading to the possibility that the C pool of karst soils is still decomposed.

5. Conclusions

The SOC and TN contents of karst forests in the karst region of southwest China were much higher than those of non-karst forests, although the C:N ratio was lower. The greater contents of SOC and TN in karst forests were mainly attributed to the higher soil Ca content and microbial biomass. In contrast, SOC and TN in non-karst forests were mainly influenced by iron oxides, pH, and TP. Additionally, compared to non-karst forests, karst forests' SOC and TN contents were less sensitive to MAT. Ca-mediated (including exchangeable Ca^{2+} and $CaCO_3$) stabilization of SOM and soil macroaggregates formed by fungal hyphae can constrain the decomposition of SOC and TN induced by warming in karst forests. Thus, relative to non-karst forests, karst forests have larger SOM pools in soils and are less sensitive to temperature change. However, due to lower C:N ratio and its higher temperature sensitivity in karst forests, climate warming may exacerbate microbial C limitation in karst forests, which may be detrimental to soil C sequestration. Based on these results, the ratio of SOC to TN (C:N) is regulated by appropriate management measures during karst vegetation restoration, thus promoting long-term stable sequestration of soil carbon pools.

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