Shrub encroachment leads to accumulation of C, N, and P in grassland soils and alters C:N:P stoichiometry – a meta-analysis

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Abstract

Soil C:N:P stoichiometry is a sensitive indicator for nutrient balance. Shrub encroachment into grasslands could change the concentrations and stoichiometry of soil carbon (C), nitrogen (N), and phosphorus (P), but the general patterns of these changes remain unclear. With a meta-analysis of a global dataset covering 344 observations from 68 studies, we examined how grassland soil C:N:P stoichiometry responds to shrub encroachment under various environmental conditions. The magnitude of the impact of shrub encroachment on soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) concentrations and their stoichiometry varied with climate, soil texture type, and soil layer. Overall, shrub encroachment significantly increased the concentrations of soil C (+29%), N (+25%), and P (+20%), and it significantly increased soil C:N (+5%), C:P (+12%), and N:P (+6%). Increases in SOC and TN concentrations mainly occurred in dry sub-humid and semi-arid zones. Regarding different soil layers, the increases in SOC and TN concentrations and in the C:N, C:P, and N:P ratios with shrub encroachment were greater in the topsoil (0-20 cm depth) than in deeper soil layers. The concentrations of SOC, TN, and TP all increased significantly with shrub encroachment in the soil texture types, such as clay, sandy loam, and sand. Shrub encroachment increased C:N, C:P, and N:P in acidic and neutral soils. The magnitude of the change in soil C:N was negatively correlated with the duration of shrub encroachment, an effect linked to a greater increase in soil TN than in SOC concentrations with longer durations of encroachment. Our global meta-analysis indicates that shrub encroachment into grasslands increases soil C sequestration and soil C:N, C:P, and N:P. Soil stoichiometric shifts in shrub-encroached grasslands are relatively sensitive to environmental factors, such as soil texture, soil pH, and climate. These findings help us to better understand the effects of shrub encroachment on biogeochemical cycling, functioning, and services in grasslands across a broad range of spatio-temporal scales.

Keywords global data analysis; shrub encroachment; soil stoichiometry; soil carbon; soil nitrogen; soil phosphorus

Introduction

Carbon (C) stocks in soils far exceed global carbon stocks in vegetation and the atmosphere combined (Lehmann and Kleber, 2015), with grasslands accounting for about one-third of all terrestrial ecosystem carbon stocks (Zhao et al., 2020). Therefore, grassland soils play an important role in the global C cycle (Guo and Gifford, 2002). Over the past century, global grasslands have been invaded by woody plants, especially shrubs, as a result of global changes such as climate warming (Ehleringer, 2005), changes in precipitation (Criado et al., 2020; Berry and Kulmatiski, 2017), elevated CO_2 concentration (Buitenwerf et al., 2012), and human activities including intentional fires (Smit et al., 2016) and overgrazing (Tjelele, 2014). The shifts from grasses to woody plants involve changes in vegetation cover (Nepstad et al., 1994; Jackson et al., 2002) and in soil C sequestration (Briggs et al., 2005). Shrub encroachment may increase C and nitrogen (N) pools in grasslands (Li et al., 2018), but some studies indicate a reduction in soil organic carbon and nitrogen content with woody plant encroachment (Jackson et al., 2002). Therefore, the role of woody plant encroachment in driving grassland C and N cycling remains largely uncertain.

Woody plants can concentrate organic matter beneath their canopies and change the microbial biomass, root biomass, litter production, soil characteristics, and microclimate (Binkley et al., 1998; Schlesinger and Pilmanis, 1998), with consequences for soil C sequestration (Connin et al., 1997) and concentration of soil total phosphorus (Mogashoa et al., 2021). Concentrations of soil organic C (SOC) tend to increase after shrub encroachment because of increases in net primary production (NPP; Boutton et al., 2009) and a lower decomposition rate for shrub litter than for grasses (Montané et al., 2010). Furthermore, N-fixing shrubs can lead to a large accumulation of available N (Buhlmann et al., 2014) and increase soil C storage (Connin et al., 1997). Ward et al. (2018) found that soil nutrient concentrations are related to shrub size rather than shrub species, and that large shrubs can increase soil nutrient concentrations in a semi-arid savanna. Soil organic C (SOC) and total nitrogen (TN) concentrations have been reported to increase with increasing shrub island size (Connell et al., 2021) but soil organic C concentration declined with increasing shrub encroachment age (Brantley et al., 2010).

Moreover, the impacts of shrub encroachment on soil total carbon (TC) and total nitrogen (TN), and soil total phosphorus (TP) concentrations can be strongly dependent on the climate and soil conditions. For example, edaphic properties significantly affect the size, distribution density, and pattern of shrubs in arid and semi-arid systems (Hughes et al., 2006). In addition, soil nutrient concentrations are related to soil texture, which can provide physical protection of organic matter inputs (Boutton et al., 2009). Moreover, Jackson et al. (2002) compared the C and N budgets of six pairs of shrub-encroached and unencroached grasslands along an annual precipitation gradient of 200 to 1100 mm and found a negative correlation between precipitation and changes in soil C and N under shrub-encroached conditions. However, here are still many different opinions on which soil texture and climate is conducive to the sequestration of soil nutrients.

Ecological stoichiometry (i.e. element ratios) provides a framework for understanding the relationship between between individual elements and how elements are coupled by exploring nutrients cycling in the form of element ratios (Sterner and Elser, 2002). Urbina et al. (2020) found that shrub encroachment slows biogeochemical cycling through the change in plant and soil stoichiometry. C:N:P stoichiometry could be used as a major indicator for soil nutrient limitation and availability, helping to explain crucial ecological processes (Cleveland and Liptzin, 2007; Reich and Oleksyn, 2004). For example, the soil C:N ratio could reflect how the decomposition rate of organic matter responds to shrub encroachment, thus enabling predictions of changes in the soil C and N cycles (Wang and Yu, 2008). In addition, ecosystem N and P status and availability may mediate ecosystem responses to climate change (Wieder et al., 2015; Terrer et al., 2019). Previous studies have shown that soil C, N, and P concentrations can respond to woody plant encroachment, but few studies have involved quantifying the responses of C:N:P stoichiometry to shrub encroachment. The impacts of shrub encroachment on soil C:N:P stoichiometry could be strongly influenced by the climate and soil conditions. For example, edaphic properties significantly affect the size, distribution density, and pattern of shrubs in arid and semi-arid systems (Hughes et al., 2006), leading to changes in soil C:N. The accurate evaluation and prediction of ecosystem structure and function require a comprehensive investigation of the influence of shrub encroachment on soil C:N:P stoichiometry.

Here, we conducted a meta-analysis with 344 observations from 68 studies of grasslands with a global distribution. The objectives of our meta-analysis were: (1) to assess the responses of soil C, N, and P concentrations and stoichiometry to shrub encroachment and (2) to explore whether such responses are mediated by shrub species, soil factors, and climatic factors in shrub-encroached grasslands. We hypothesized that: (1) shrub encroachment would increase soil C, N, and P concentrations and thus alter element stoichiometry in grasslands; and (2) the responses of soil nutrient concentrations and stoichiometry to shrub encroachment would depend on shrub species identity and site environmental factors (i.e., mean annual precipitation, soil pH, soil depth, and soil texture).

Materials and methods

Data collection and extraction

We set up a comprehensive database of responses of soil nutrient concentrations and stoichiometry to woody plant encroachment from published global literature. First, we searched the Web of Science and Google Scholar databases for online papers published before July 2022. The combined search terms used in the database were '(either shrub, bush, woody, shrubification, grassland, steppe, prairie, savanna, or shrubland) and (either encroachment, expansion, thickening, proliferation, or colonization) and (soil carbon, nitrogen, phosphorus, nutrient, stoichiometry, or C, N, P, C:N, C:P, N:P, C:N:P)' (Table S1 in Appendix S1). Then, we included those studies which satisfied the following criteria: (1) only experiments conducted in grassland ecosystems were included, i.e., those from tundra, forest, desert, and wetland were excluded; (2) shrub encroachment was a natural process, without any artificial introduction, and field experiments were conducted under natural conditions; (3) shrub-encroached grassland was treated as one landscape type, and the variables were compared before and after shrub encroachment (i.e., treatment and control); (4) sample size, mean values, and standard deviations or errors of variables were reported.

Based on these criteria, we included 344 cases in our database, derived from 68 studies (Fig. 1, Table S2 in Appendix S1). Among the 344 independent plot-level cases, 39% were from North America, 25% were from Asia, 11% were from Europe, 20% were from Africa, 3% were from South America and 2% were from Australia. More than half of the studies were from savanna, while the rest were from semi-arid grassland, arid grassland, and mesic grassland. All studies were from tropical and mid latitudes. The latitudes ranged from 43°S to 46.56°N and the longitudes from 110.88°W to 145.43°E; the mean annual precipitation was between 134 and 1559 mm, the mean annual temperature was between 1.1 and 24°C, and the average altitude was between 75 and 3500 m.

For each publication, we collected the basic geographical information of the experiment (site location, continent, latitude and longitude, landscape type, precipitation, temperature, duration of shrub encroachment), the identity and basic traits of the soil (pH, depth, texture), the taxonomic identity of the dominant encroaching woody species (species, genus, and family), and the mean, standard deviation, and sample size of the ecosystem responses that were assessed in plots with and without woody plant encroachment. We extracted data on soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) from the text or tables of each publication. We extracted data from published figures using the GetData Graph Digitizer software (version 2.26, http://getdata-graph-digitizer.com). In most publications, only the response of element concentrations or element stocks to shrub encroachment was reported. For our study, we calculated soil C:N, N:P, and C:P as molar ratios. For publications where only soil organic matter concentration was reported, we calculated soil total carbon (TC) concentration as soil organic matter concentration divided by a constant of 1.724.

Data analysis

We defined the individual effect of shrub encroachment as the response of a specific variable (e.g., C:P ratio) when compared with the control, that is, the grassland without shrub encroachment, and we defined the grassland encroached by shrubs as the experimental group. We evaluated the responses of soil C, N, and P concentrations and their stoichiometry to shrub encroachment following the methods of Gurevitch and Hedges (1999), with lnRR defined as the "effect size". We conducted the meta-analysis with the MetaWin 2.1 software (Rosenberg et al., 2000). For each pair of soil variables from sites with and without encroachment, we calculated the response ratio of soil C, N, and P concentrations and their stoichiometry as a measurement of effect size (Rosenberg et al., 2000).

$$\mathrm{lnRR} = \ln\left(\frac{\mathrm{X_e}}{\mathrm{X_c}}\right) \tag{1}$$

where X_e and X_c are the means of variables from the experimental and control groups, respectively. Positive and negative lnRR values indicate that shrub encroachment increases and decreases element ratios compared with the control, respectively.

We estimated the variance (V) associated with lnRR from the standard deviation of each mean value (Koricheva et al., 2013):

$$\mathrm{V} = \frac{(\mathrm{SD_e})^2}{\mathrm{n_e X_e}^2} + \frac{(\mathrm{SD_c})^2}{\mathrm{n_c X_c}^2} \tag{2}$$

where SD_e and SD_c are the standard deviations of variables from the experimental and control groups, respectively; X_e and X_c are the means of variables from the experimental and control groups; and n_e and n_c are the sample sizes of variables from the experimental and control groups. We calculated the standard deviation of nutrient concentrations from the standard error if there only standard error was reported in a publication. We estimated the standard deviation of element ratios based on similar experimental studies (Gao et al., 2021).

We divided the soil into layers by depth: 0–20 cm depth was defined as the topsoil layer, 20–60 cm as the middle soil layer, and 60–100 cm as the bottom soil layer. These layers corresponded approximately to the A, B, and C horizons of each soil. We classified the sites into four climate zones according to mean annual precipitation (MAP;

Middleton and Thomas, 1997): humid (> 800 mm), dry sub-humid (400–800 mm), semi-arid (200–400 mm), and arid (0–200 mm). In addition, we classified the soil samples into three pH categories: acidic (pH < 6.5), neutral (pH between 6.5 and 7.5), and alkaline (pH > 7.5). Following the USDA taxonomy system (Brown, 1998; Staff, 1993), we further classified the soils in our database into nine texture types: sand, sandy loam, loam, silty loam, sandy clay loam, clay loam, sandy clay, silty clay, and clay. There were 23 shrub genera in our database: *Acacia*, *Caragana*, *Genista*, *Prosopis*, *Schotia*, *Senegalia*, *Brachystegia*, *Chuquiraga*, *Cistus*, *Echinospartum*, *Eremophila*, *Juniperus*, *Larrea*, *Morella*, *Nassella*, *Potentilla*, *Quercus*, *Rosmarinus*, *Artemisia*, *Salix*, *Symphoricarpos*, *Spiraea*, and *Vaccinium*, which we classified into two groups: Leguminosae and non-Leguminosae.

Several factors, such as climate (precipitation), soil properties (pH, depth, texture), dominant plant (shrub genus, shrub families), and identity of encroachment (i.e., history of shrub encroachment) were expected to influence the relationship between nutrient status and shrub encroachment. We therefore considered these factors as moderator variables in our meta-analysis.

We evaluated the overall effect of shrub encroachment on soil C, N, and P concentrations and stoichiometry using a weighted random-effects model for meta-analysis (Gurevitch and Hedges, 1999). We used Equation (3) to derive the weighted mean response ratio (lnRR_{++}).

$$\mathrm{lnRR}_{++} = \frac{\sum_{i=1}^{m}\sum_{j=1}^{k} w_{ij}\mathrm{lnRR}_{ij}}{\sum_{i=1}^{m}\sum_{j=1}^{k} w_{ij}} \qquad (3)$$

where lnRR_{++} is the weighted mean response ratio, m is the group number (e.g., soil texture), k is the comparative number of ith group, and w is the weight of the response ratio. Based on 999 iterations, we calculated the 95% confidence intervals (CI) of lnRR_{++}. We considered an experimental effect significant ($P < 0.05$) if its 95% CI did not include zero (Koricheva et al., 2013).

We measured the total heterogeneity across studies using the Q_t statistic (Table S1 in Appendix S2). There was significant residual heterogeneity in the random effects for the SOC concentration (Q_t=1066.3, $P < 0.0001$), TN concentration ($Q_t = 416$, $P <$

0.0001), and TP concentration ($Q_t = 252.0$, $P < 0.0001$) datasets, and for the C:N ($Q_t = 292.0$, $P < 0.0001$), C:P ($Q_t = 381.7$, $P < 0.0001$), and N:P ($Q_t = 381.7$, $P < 0.0001$) datasets, which we tried to explain with different moderators. We thus analyzed five subgroups within the studies, i.e., climate classes, shrub families and species, soil layers, soil textures, and soil pH categories.

The normal P-P plot (Fig. S2 in Appendix S2) showed that the effect sizes were normally distributed. We assessed publication bias of each variable in our database with funnel plots (Rosenberg et al., 2000). The funnel plots combined with the P values (> 0.05) based on an Egger's regression test showed no publication bias (Fig. S1 in Appendix S2). The stability of an effect size increases and the size of the confidence interval decreases with increasing sample size, which meant our large database yielded accurate results. We tested the relationships among soil nutrient concentrations and stoichiometric ratios using a Pearson correlation analysis. Moreover, we conducted a linear regression analysis to explore the relationship between continuous variables (duration of shrub encroachment) and changes in stoichiometric ratios. We did not conduct this linear regression analysis when fewer than 30 studies were included. We used SPSS 25.0 (SPSS Inc., Chicago, IL, USA) for both the Pearson correlation analysis and the linear regression analysis.

Results

Effects of climate and soil properties on soil C, N, and P concentrations and their stoichiometry

For the full database, the concentrations of SOC, TN, and TP increased significantly after shrub encroachment, by 29%, 25%, and 20%, respectively ($P < 0.05$; Fig. 2a, b, c). SOC concentration in the semi-arid, dry sub-humid, and humid zones increased by 33%, 36%, and 4%, respectively ($P < 0.05$), while no SOC response was detected in the arid zone. SOC concentration were significantly enhanced in the topsoil layer, by 39%, after shrub encroachment. Furthermore, shrub encroachment increased SOC concentration in clay, silty clay, sandy clay loam, sandy loam, and sand, by 36%, 40%, 32%, 36%, and 35%, respectively, but not in the other soil textures. SOC concentration increased significantly, by 56%, with shrub encroachment in sites with neutral soil (Fig. 2a).

The response of soil TN concentration to shrub encroachment was only significant in the dry sub-humid (+25%), semi-arid (+23%), and humid zones (+22%). Meanwhile, TN concentration responded significantly to shrub encroachment in the topsoil and middle soil layers, increasing by 29% and 13%, respectively, but did not show a response in the bottom soil layer. TN concentration increased significantly with shrub encroachment only in the clay, sandy loam, and sand soil textures, by 23%, 32%, and 29%, respectively. TN concentration increased significantly in all soil pH categories, by 27% (acidic), 40% (neutral), and 17% (alkaline) (Fig. 2b).

TP concentration increased significantly in the humid (45%) and dry sub-humid (21%) zones, but not in the semi-arid and arid zones. Similar to TN, TP concentration increased significantly in the topsoil and middle soil layers, by 27% and 15%, respectively, but did not respond significantly in the bottom soil layer. TP concentration also increased significantly with shrub encroachment into clay, sandy loam, and sand, by 128%, 13%, and 26%, respectively, but showed no significant responses in the other soil texture s. The response of TP concentration to shrub encroachment was only significant in acidic soil, where values increased by 32% (Fig. 2c).

Soil C:N, C:P, and N:P increased by 6%, 7%, and 7%, respectively, after shrub encroachment ($P < 0.05$; Fig. 2d, e, f). Soil C:N increased significantly in the dry sub-humid, semi-arid, and humid zones, by 6%, 3%, and 4%, respectively ($P < 0.05$), but no significant response was detected in the arid zone. Shrub encroachment reduced soil C:N by 11% in the bottom soil layer, but increased this ratio significantly in the topsoil and middle soil layers ($P < 0.05$). The responses of soil C:N varied across soil textures, with an enhancement in clay (18%), sandy clay loam (11%), loam (5%), and sandy loam (4%) but no significant response in sandy clay, clay loam, and sand. Soil C:N increased significantly in acidic and neutral soils, but there was no significant response in alkaline soil (Fig. 2d).

Soil C:P only increased significantly with shrub encroachment in the dry sub-humid zone (by 12%). In addition, soil C:P increased in the topsoil layer (by 19%) and in sandy loam (by 29%) after shrub encroachment, but no significant response was detected in other soil layers or soil textures. Additionally, soil C:P increased significantly in acidic and neutral soils, by 18% and 29%, respectively, but no significant response was observed in alkaline soil (Fig. 2e).

Shrub encroachment reduced soil N:P in the humid zone, by 33%, but increased this ratio in the dry sub-humid (8%) and semi-arid (30%) zones. Soil N:P only responded significantly in the topsoil layer, with an increase of 12%. In addition, shrub encroachment increased soil N:P significantly (by 23%) in sandy loambut decreased significantly (by 34%) in clay, while no response was detected in other soil textures. Shrub encroachment enhanced soil N:P in acidic and neutral soils, by 7% and 16%, respectively, while no significant effect was observed in alkaline soil (Fig. 2f).

Effects of shrub identity on soil C, N, and P concentrations and their stoichiometry

SOC concentration increased significantly when encroached by Leguminosae and non-Leguminosae, by 39% and 30%, respectively. Regarding leguminous shrubs, SOC concentration increased significantly with encroachment by *Caragana*, *Genista*, and *Prosopis*, by 17%, 39%, and 52%, respectively (Fig. 3a). For non-leguminous shrubs, SOC concentration increased significantly with encroachment by the genera of *Morella* (+140%), *Potentilla* (+36%), *Rosmarinus* (+44%), *Artemisia* (+100%), and *Vaccinium* (+166%; Fig. 3a). TN concentration were enhanced significantly when encroached by Leguminosae and non-Leguminosae, by 25% and 18%, respectively. Specifically, TN concentration increased significantly when encroached by *Acacia*, *Prosopis*, *Morella*, *Potentilla*, *Quercus*, and *Vaccinium*, by 24%, 34%, 133%, 18%, 24%, and 94%, respectively (Fig. 3b). Overall, TP concentration increased significantly when encroached by Leguminosae and non-Leguminosae, by 16% and 35%, respectively. TP concentration only increased significantly (by 128%) when the encroaching genus was *Vaccinium* (Fig. 3c).

Overall, soil C:N increased significantly with encroachment by Leguminosae and non-leguminosae, by 4% and 6%, respectively. Specifically, when encroached by the leguminous genera *Caragana* and *Prosopis*, C:N increased by 4%. Soil C:N also increased significantly when encroached by the non-leguminous genera of *Brachystegia* (+15%), *Potentilla* (+7%), *Quercus* (+4%), *Symphoricarpos* (+13%), and *Spiraea* (+9%; Fig. 3d). Non-leguminous shrubs enhanced soil C:P by 31% overall, while leguminous shrubs did not affect this ratio. Soil C:P decreased significantly, by 18%, with encroachment by the leguminous *Caragana* genus. In contrast, soil C:P increased significantly with *Prosopis* (leguminous) and *Potentilla* (non-leguminous) encroachment, by 22% and 34%, respectively (Fig. 3e). Overall, soil N:P increased significantly, by 13%, with encroachment by non-leguminous shrubs, but not by leguminous shrubs. Regarding individual genera, soil N:P decreased with the encroachment of *Caragana* (leguminous) and of *Echinospartum*, and *Vaccinium* (non-leguminous), by 20%, 32%, and 34%, respectively. Furthermore, soil N:P increased by 24%, 18%, and 43% when encroached by the genera *Prosopis*, *Potentilla*, and *Salix*, respectively, but it showed no significant change with the other encroaching shrub genera (Fig. 3f).

Soil C, N, and P concentrations and their stoichiometry in relation to shrub encroachment duration

The changes in SOC and TN concentrations were positively correlated with the duration of shrub encroachment (both $P < 0.001$; Fig. 4a, b), whereas the changes in soil C:N were negatively correlated with the duration of shrub encroachment ($P = 0.0017$; Fig. 4c). There were negative correlations between soil P concentration and C:P, between P concentration and N:P, and between C:N and N:P (all $P < 0.01$; Table S2 in Appendix S2). SOC concentration was positively correlated with P concentration, C:N, and C:P (all $P < 0.01$). Similarly, soil N concentration was positively correlated with P concentration ($P < 0.01$), C:N ($P < 0.05$), C:P ($P < 0.01$), and N:P (all $P < 0.01$). Additionally, there were negative correlations between soil P concentration and C:P and N:P, and a positive correlation between soil P concentration and C:N (all $P < 0.01$).

Discussion

Responses of soil nutrient concentrations and stoichiometry to shrub encroachment

SOC, TN, and TP concentrations and their stoichiometric ratios changed in response to shrub encroachment. There are at least five possible reasons for such responses. First, shrubs are less nutrient conservative than herbs (Elser et al., 2000; Thompson et al., 1997), leading to greater C and N storage. In particular, shrub litter is more recalcitrant than grass litter (Montané et al., 2010) due to higher concentrations of C-rich recalcitrant biopolymers. For instance, the litter and roots of woody plants have high levels of cinnamyl and other compounds that are resistant to mineralized, thus being harder to decompose (Opsahl and Benner, 1997). Second, higher litter quantities and NPP in shrub-encroached grasslands (Ding et al., 2019) can increase SOC storage (Boutton et al., 2009). Third, shrub encroachment enhances the soil retention capacity and reduces leaching and runoff, which can improve soil P retention (Ding et al., 2019). Fourth, shrubs with deeper root systems deposit more C in the soil (Wang et al., 2016) than occurs in grasslands without shrubs. Fifth, the fixation of N by leguminous shrub can enhance soil N concentration (Su and Zhao, 2003). Shrub encroachment is conducive to the sequestration of C and N, but the disproportionate increases in C and N may lead to an imbalance in soil C:N. In addition, the relationship between soil C:N stoichiometry and microbial activity may affect C sequestration, as microbes have a lower C use efficiency when the C:N ratio is high (> 15) (Alberti et al., 2014). Increases in soil C:N after shrub encroachment may additionally enhance soil N availability (Alberti et al., 2014).

The role of climatic factors

The increases in soil SOC and N with shrub encroachment reached a maximum in dry sub-humid and semi-arid climate zones, suggesting that medium precipitation levels are good for C sequestration during shrub encroachment. Simultaneously, soil C:N and N:P increased significantly in dry sub-humid and semi-arid grasslands, and C:P also increased significantly in the dry sub-humid climate zone. Soil C:N:P stoichiometry is strongly effected by soil moisture, which is altered strongly by precipitation intensity (Sun et al., 2020; Yuan and Chen, 2015). Previous studies have indicated that soil C and N concentrations in shrub-encroached grasslands may increase (Wheeler et al., 2007) or decrease (Jackson et al., 2002) with increasing precipitation. Changes in precipitation could significantly influence the wetting-drying cycle of soil, litter mass (Denef et al., 2001), and the activities of microorganisms (Xu et al., 2021). Soil microbial activity increases considerably with high annual precipitation in general, which can have a significant effect on litter decomposition rate, thus resulting in changes in the use of soil nutrients (Denef et al., 2001) and in the nutrient limitation situation. In our study, soil N:P increased significantly with shrub encroachment in the semi-arid zone, as the increase in N far exceeded the increase in P, which may have resulted in a P limitation in the soil. In contrast, we found that the changes in soil C:N after shrub encroachment were similar along the precipitation gradient, indicating that the response of soil C:N to shrub encroachment is less sensitive to variation in climate.

The role of soil conditions

The changes in C, N, and P concentrations were much stronger in the topsoil layer than in deeper layers, which is similar to the finding of Boutton et al. (2009). This may be because the SOC in topsoil mainly comes from leaf litter and root exudates (Wu et al., 2023), and the greater amount of litter input into the topsoil after shrub encroachment (as shrubs have a greater biomass) enhances C accumulation in the topsoil. The roots of shrubs are mainly distributed in the 0–60 cm depth soil layer, so the bottom soil layer (60–100 cm depth) receives less leaf litter and root exudates compared with the topsoil, explaining why there was no significant increase in SOC in the bottom soil layer. Soil C:N, C:P, and N:P increased significantly in the topsoil because the increases in SOC and TN concentrations in the topsoil were greater than that of TP. This may be caused by the relatively simple input path of P (Schlesinger and Bernhardt, 2020). Our results demonstrate that there exists a strong edaphic control of soil nutrient element accumulation and soil stoichiometry associated with shrub encroachment. Soil texture has been reported to significantly affect the woody-to-grass ratio, due to its effects on soil water content, plant growth, and nutrient concentrations and availability (Britz and Ward, 2007). We found that the concentrations of SOC, TN, and TP, as well as C:N, increased with shrub encroachment in clay soil. Physical protection of SOC varies with soil structure. Liao et al. (2006) reported that about 30–55% of the SOC generated after shrub encroachment was protected within macro- and microaggregates or correlated with the fraction of silt and clay. Fine-textured soil (higher silt and clay contents) is characterized by organomineral complexes and the formation of macro- and microaggregates, which protect organic matter from mineralization and/or eluviation (Campbell et al., 1998) and from decomposition by soil microbes (Ladd et al., 1993; Christensen, 2020). In our meta-analysis, soil nutrient element concentrations increased significantly after shrub encroachment in sandy soil, which may be because coarse-textured soils tend to have a higher bulk density (Håkansson and Lipiec, 2000). Meanwhile, soil N:P responded negatively in clay soil, which may be attributed to the fact that the magnitude of TP increase far exceeded the magnitude of TN increase.

Acidic soil affects the decomposition rate of litter and the release rate of soil C and N (Hättenschwiler and Bretscher, 2010). Moreover, the C storage in acidic soil is greater under high N conditions than under low N conditions (Hagedorn et al., 2003). Consistent with these previous findings, we observed that SOC and TN increased in acidic soil after shrub encroachment, especially by Leguminosae shrubs. In neutral soil, C:N, C:P, and N:P increased significantly, which may be attributed to the large increases in TN and especially SOC concentration after shrub encroachment in neutral soil, combined with the smaller change in TP concentration (Fig. 2a, b, c).

The role of woody plant type

Shrub type could affect soil nutrient concentrations by altering litter quality and nutrient uptake. The spread of N-fixing shrubs leads to massive reactive nitrogen enrichment (Bühlmann et al., 2014) and may be an important driving factor in the increases in SOC observed here. The encroachment of non-leguminous shrubs led to a significant increase in C:P in our meta-analysis, reflecting the lower availability of P (Wang and Yu, 2008). A greater increase in soil C:P in grasslands encroached by non-leguminous shrubs therefore results in a decrease in available P that can be absorbed by plants for growth. Leguminous shrub encroachment did not affect soil C:P significantly, reflecting a non-significant effect on available P.

Conclusions

In summary, our global synthesis verifies that shrub encroachment promotes soil C sequestration and increases soil C:N, C:P, and N:P. Moreover, SOC and TN accumulate over time after shrub encroachment. Most importantly, shrub encroachment simultaneously shifts soil stoichiometry in grasslands with sandy loam, acidic, and neutral soils, as well as in those with a dry sub-humid climate. The rate of increase in soil C:N decreases with the duration of shrub encroachment, suggesting a stabilization of the soil C:N ratio in shrub-encroached grasslands. These findings provide fundamental knowledge for estimating the impacts of shrub encroachment (partly due to global climate change) on C and N cycling in grasslands, and they help to better predict the effects of global change on grassland biogeochemical cycling and ecosystem services in a changing world.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

This study was conceived by Z.D. and H.Z. The data was collected and analyzed by H.Z., D.D., X.C., D.G., Y.H., and S.N. The manuscript was written by Z.D. and H.Z., with input from J.P., J.S., X.L. and M.L. and comments from all other authors.

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