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# 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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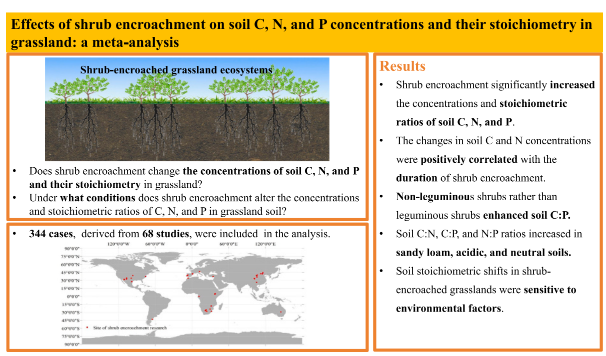
## HIGHLIGHTS

- Shrub encroachment significantly increased the concentrations and stoichiometric ratios of soil C, N, and P.
- The changes in soil C and N concentrations were positively correlated with the duration of shrub encroachment.
- Non-leguminous shrubs rather than leguminous shrubs enhanced soil C:P.
- Soil C:N, C:P, and N:P ratios increased in sandy loam, acidic, and neutral soils.
- Soil stoichiometric shifts in shrub-encroached grasslands were sensitive to environmental factors.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Soil stoichiometry of carbon (C), nitrogen (N), and phosphorus (P) are indicators for nutrient balance. Shrub encroachment into grasslands could change nutrient concentrations and stoichiometry in soils, but the general patterns remain unclear. With a meta-analysis of a global dataset covering 344 observations from 68 studies, we examined the responses of grassland soil C:N:P stoichiometry to shrub encroachment under various environmental conditions. Our results show that: 1) Shrub encroachment significantly increased the concentrations of soil C (+29 %), N (+25 %), P (+20 %), C:N (+5 %), C:P (+12 %), and N:P (+6 %). The magnitude of such effects varied with climate, soil texture, and soil layer. 2) Increases in SOC and TN concentrations mainly occurred in Mediterranean and very humid climate zones. Soil C:P and N:P decreased in semi-humid climate zone after shrub

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Soil nitrogen
Soil phosphorus

encroachment. 3) The increases in SOC and TN concentrations and in the C:N, C:P, and N:P ratios after shrub encroachment were greater in the topsoil than in deeper soil layers. 4) Both finest-textured soil (clay) and coarsest-textured soil (sand) are beneficial for increase of soil nutrient concentrations following shrub encroachment. 5) The magnitude of the change in soil C:N was negatively correlated with the duration of shrub encroachment, due to greater increases in soil TN than in SOC concentrations with longer durations of encroachment. Our results indicate that soil stoichiometric shifts in shrub-encroached grasslands are relatively sensitive to environmental factors, including 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.

## 1. 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 and Giardina, 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 (Bühlmann 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 and Young, 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 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). And there are significant correlations between the ratio of soil C/P, soil N/P and soil microbial properties (Shen et al., 2018). 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 C:N, C:P, and N:P to shrub encroachment could be significantly affected by "local" conditions including climate, soil pH, soil depth, and soil texture.

## 2. Materials and methods

### 2.1. 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.

### 2.2. 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).

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

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):

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

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).

Aridity indices classify the climate types based on the availability of water resources (Moral et al., 2016). We measure aridity with De Martonne aridity index ($I_{DM}$) (Croitoru et al., 2013):

$$I_{DM} = \frac{P}{T_a + 10} \tag{3}$$

where *P* is the mean annual precipitation (in millimeters) and *Ta* is the mean annual air temperature (in degree Celsius). We classified the sites

**Table 1**
Type of climate according to the de Martonne aridity index ($I_{DM}$, adapted after Baltas, 2007).

| Climate type | $I_{DM}$ values |
|---|---|
| Arid | $I_{DM}$<10.0 |
| Semi-arid | 10.0≤$I_{DM}$<20.0 |
| Mediterranean | 20.0≤$I_{DM}$<24.0 |
| Semi-humid | 24.0≤$I_{DM}$<28.0 |
| Humid | 28.0≤$I_{DM}$<35.0 |
| Very humid | 35.0≤$I_{DM}$≤55.0 |
| Extremely humid | $I_{DM}$>55.0 |

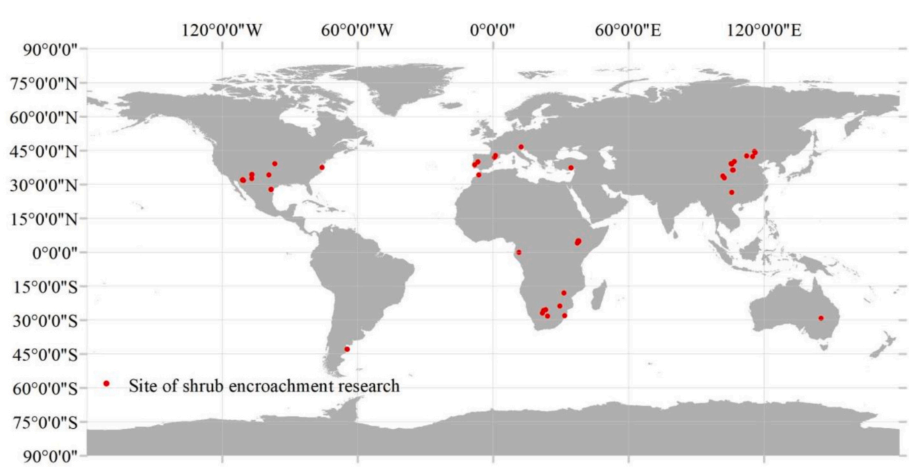


**Fig. 1.** Geographical locations of the 68 studies included in the meta-analysis.
(Note: It can be seen from the map that the number of research points is <68, because of the problem of map resolution, the research points in many articles overlap).

into seven climate zones according to aridity index (Table 1).

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. 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 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 were further classified into two groups: Leguminosae and non-Leguminosae.

Several factors, such as climate, 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 Eq. (4) to derive the weighted mean response ratio ($\ln RR_{++}$):

$$\ln RR_{++} = \frac{\sum_{i=1}^{m}\sum_{j=1}^{k} w_{ij} \ln RR_{ij}}{\sum_{i=1}^{m}\sum_{j=1}^{k} w_{ij}} \tag{4}$$

where $m$ is the group number (e.g., soil texture), $k$ is the comparative number of $i$th group, and $w$ is the weight of the response ratio. Based on 999 iterations, we calculated the 95 % confidence intervals (CI) of $\ln RR_{++}$. 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.

## 3. Results

### 3.1. General responses of soil C, N, and P concentrations and stoichiometry

For the full database, the concentrations of SOC, TN, and TP significantly increased by 29 %, 25 %, and 20 % after shrub encroachment, respectively ($P < 0.05$; Fig. 2a, b, c). For the climate zone, SOC concentration only increased significantly in the Mediterranean and very humid zones ($P < 0.05$). For the soil layer, the highest increase in SOC concentration in the topsoil layer after shrub encroachment is 39 %. Furthermore, shrub encroachment increased SOC concentration in clay, silty clay, sandy clay loam, sandy loam, and sand, but not in the other soil textures. The concentrations of SOC were increased in acidic, neutral soil, and alkaline soil (Fig. 2a).

The responses of soil TN concentration to shrub encroachment were only significant in the Mediterranean (+35 %), very humid (+31 %), and extremely humid climate zones (+28 %). Meanwhile, TN concentration responded significantly to shrub encroachment in the topsoil and middle soil layers, but not in the bottom layer. TN concentration increased significantly with shrub encroachment only in the clay, sandy loam, and sand soil textures, by 23 %, 32 %, and 29 %, respectively, no significant changes observed in other soil textures. TN concentration increased significantly in all soil pH categories (Fig. 2b).

TP concentration increased significantly in the Mediterranean (17 %), semi-humid (131 %), very humid (27 %) and extremely humid climate zones (+15 %), but not in the semi-arid and arid zones. Similar to TN, TP concentration increased significantly in the topsoil and middle soil layers, but not in the bottom layer. The TP concentration also significantly increased with the encroachment of shrubs into clay, sandy loam, and sand, especially increasing by 128 % in clay, but showed no significant responses in the other soil textures. Shrub encroachment did not affect TP in neutral and alkaline soils, but increased that in acidic soil 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 semi-arid, semi-humid, and extremely humid climate zones, by 7 %, 16 %, and 7 %, respectively ($P < 0.05$), but no significant response was detected in the arid, Mediterranean, and very humid climate zones. Shrub encroachment reduced soil C:N in the bottom soil layer, but increased that 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 very humid (by 20 %) and extremely humid climate zones (by 31 %), but decreased significantly in semi-humid climate zone by 109 %. 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, but no significant response was observed in alkaline soil (Fig. 2e).

Shrub encroachment reduced soil N:P in the semi-humid climate zone, by 150 %, but increased this ratio in the semi-arid (13 %) and extremely humid (120 %) climate zones. In different soil layers, 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 loam but 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, while no significant effect was observed in alkaline soil (Fig. 2f).

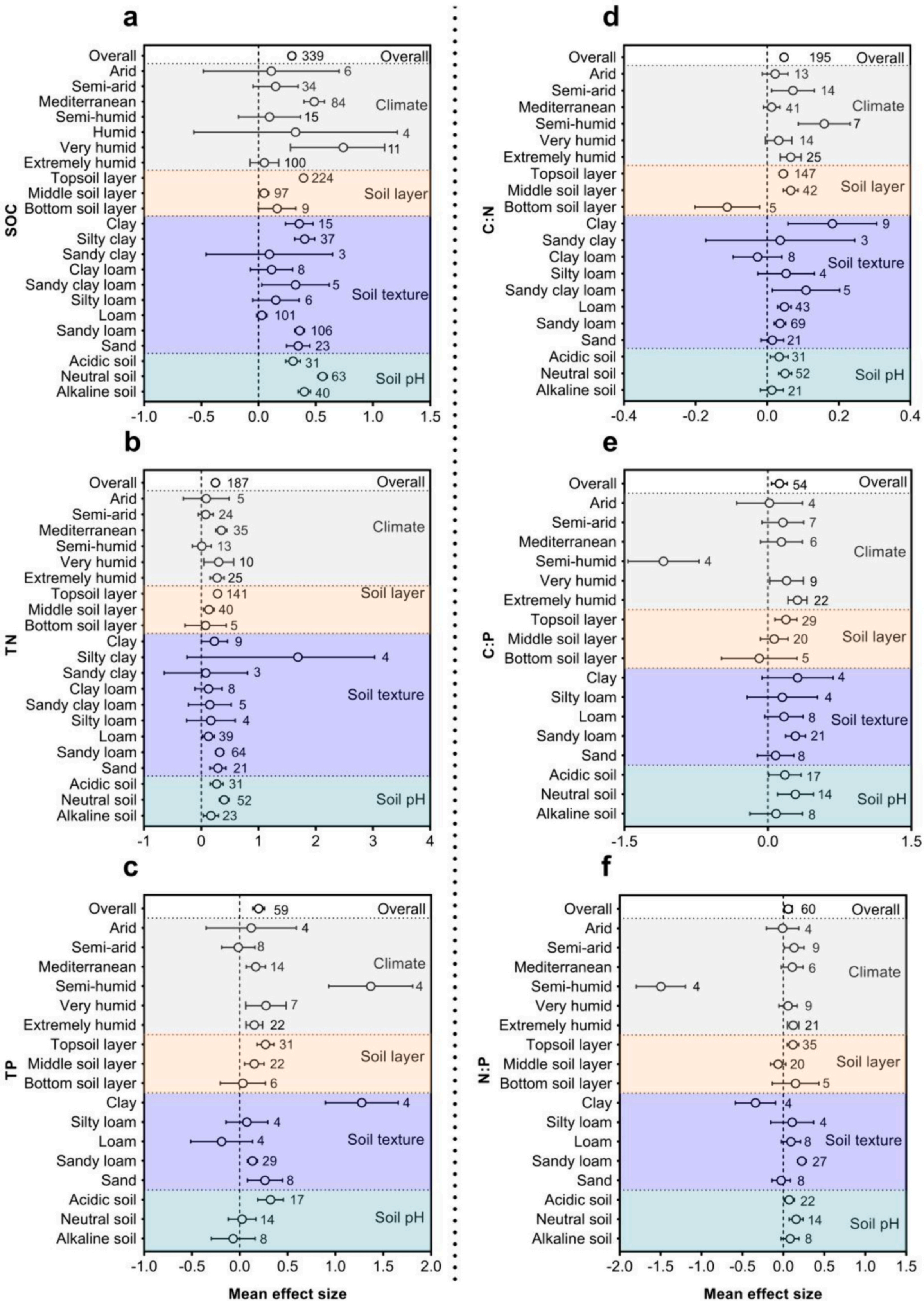


**Fig. 2.** Mean effect sizes of shrub encroachment in different environments on (a) soil organic carbon concentration (SOC), (b) soil total nitrogen concentration (TN), and (c) soil total phosphorus concentration (TP), and on the stoichiometric ratios (d) the ratio of SOC and TN (C:N), (e) the ratio of SOC and TP (C:P), and (f) the ratio of TN and TP (N:P). Error bars represent 95 % confidence intervals, and sample size numbers are shown next to the error bars. The effect of shrub encroachment was considered significant if the 95 % confidence interval did not cross zero.

### 3.2. The role of shrub identity

SOC concentration increased significantly when encroached by Leguminosae and non-Leguminosae, by 39 % and 30 %, respectively. The magnitudes of such enhancement varied across different plant genera (Fig. 3a). TN concentration was enhanced significantly when encroached by Leguminosae and non-Leguminosae, by 25 % and 18 %, respectively. Specifically, the maximum increase in soil TN concentration occurs when encroached by *Morella* (+133 %) and *Vaccinium* (+94 %), the two non-Leguminosae shrub (Fig. 3b). Overall, TP concentration

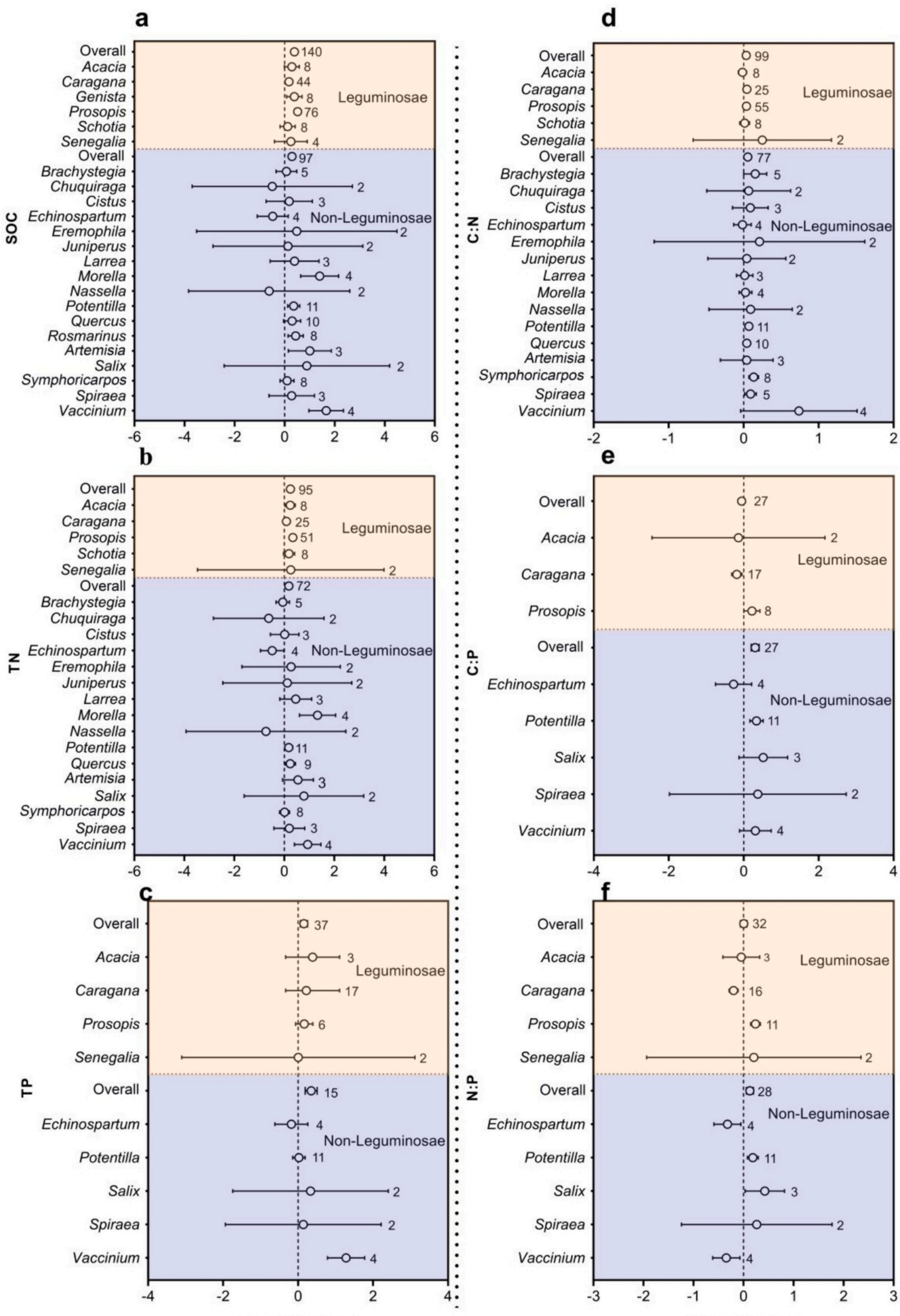


**Fig. 3.** Mean effect sizes of encroachment by different shrubs on (a) soil organic carbon concentration (SOC), (b) soil total nitrogen concentration (TN), and (c) soil total phosphorus concentration (TP), and on the stoichiometric ratios (d) C:N, (e) C:P, and (f) N:P. Error bars represent 95 % confidence intervals, and sample size numbers are shown next to the error bars. The effect of shrub encroachment was considered significant if the 95 % confidence intervals did not cross zero.

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*, *Potentilla*, *Quercus*, *Snowberry*, and *Spiraea* (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 encroached by non-leguminous shrubs, but there is no significant change when encroached 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 did not change with the other shrub genera (Fig. 3f).

### 3.3. The role of 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).

## 4. Discussion

### 4.1. Responses of soil nutrient concentrations and stoichiometry to shrub encroachment

In consistent with our first hypothesis, we found positive responses of SOC, TN, and TP concentrations and stoichiometric ratios to shrub encroachment. There are at least the following possible reasons for such responses. For the increase in SOC concentration, shrubs are less nutrient conservative than herbs (Elser et al., 2000; Thompson et al., 1997), leading to greater soil 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 decomposition (Opsahl and Benner, 1997). Higher litter quantities and NPP in shrub-encroached grasslands (Ding et al., 2019) can increase SOC storage (Boutton et al., 2009). In addition, shrubs with deeper root systems deposit more C in the soil (Wang et al., 2016) than herbs in grasslands. For the increase in TN

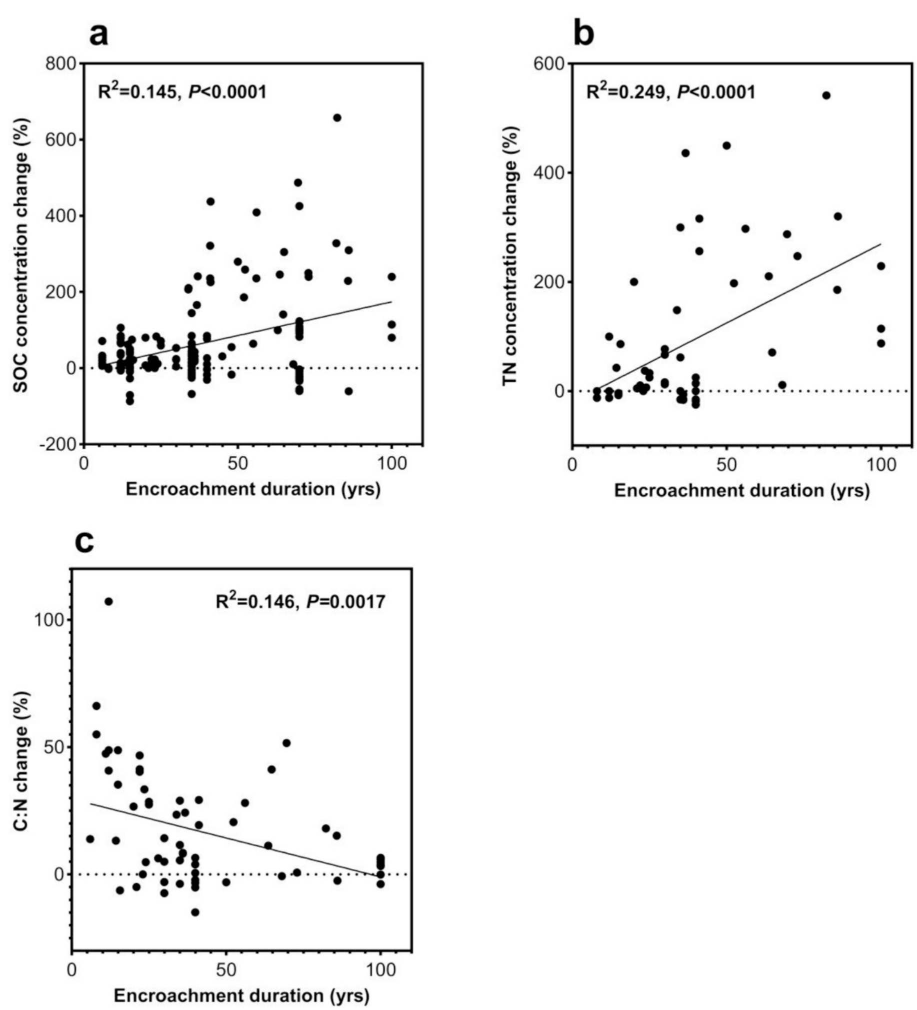


**Fig. 4.** Relationships between shrub encroachment duration and changes in (a) soil organic carbon concentration (SOC), (b) soil total nitrogen concentration (TN), and (c) soil C:N.

concentration, the fixation of N by leguminous shrub can enhance soil N concentration (Su and Zhao, 2003). For the increase in TP concentration, second, higher litter quantities and NPP in shrub-encroached grasslands (Ding et al., 2019) can increase SOC storage (Boutton et al., 2009). Shrub encroachment converted more P into non-available P (Ding et al., 2019), which lose less through runoff (Hobbie et al., 2017) and leaching (Nidzgorski and Hobbie, 2016) than water-soluble P, thereby improved soil P retention (Ding et al., 2019).

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, an increase in soil C:N stoichiometry can lead to lower microbial C use efficiency, so they respire more fresh C inputs and promote decompose soil organic matter, which enhance soil N availability (Alberti et al., 2014). The increase of soil C:P and N:P ratio due to the larger increase in soil C and N compared to P, which may influence the soil microbial community dynamics, that lead to feedbacks on soil C, N and P availability (Shen et al., 2018).

### 4.2. The role of climate

Our research showed that there are great increases of soil SOC and N with shrub encroachment in Mediterranean and very humid climate zone, suggesting that medium and high humidity level is good for soil C and N sequestration during shrub encroachment, As is well known, there is a positive correlation between humidity and annual average precipitation. Wheeler et al. (2007) also found that soil C and N concentrations in shrub-encroached grasslands may increase with increasing precipitation, which likely due to a higher biomass production (Lie et al., 2018). There are both increased of soil N and P in the climate of very humid and extremely humid, which may be due to rich water increased the concentrations of plant N and P but reduced efficiency of their absorption by plants, result in an increase in N and P return to soil via litter (Ren et al., 2015).

In our study, soil C:P and N:P decreased significantly with shrub encroachment in the semi-humid zone, as there is a significant increase in soil P, but almost no change in C and N. Which showed that the stoichiometry of soil C:N:P is affected by aridity (Jiao et al., 2016). Such divergent responses of C, N, and P may cause an imbalance of soil C, N, P, with negative impacts on plant growth and production in semi-humid ecosystems (Jiao et al., 2016). In contrast, we found that soil C:N all increased in the semi-arid, semi-humid and extremely humid zones after shrub encroachment, which may influence soil microbial community structure (Wan et al., 2014), and influence soil microbial community composition by affecting the decomposition of soil organic matter (Wan et al., 2014).

### 4.3. The role of soil layers and conditions

Our results indicated the strong variations in the responses of soil nutrient status to shrub encroachment across different soil conditions, supporting our second hypothesis. The changes in C, N, and P concentrations were much stronger in the topsoil layer than in deeper layers, which is in line with 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. There was no significant increase in SOC in the bottom soil layer, it may be because that the SOC content of 60–100 cm soil layer is not sensitive of different land uses (Huang et al., 2017). 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 a strong edaphic control of soil nutrient element accumulation and soil stoichiometry associated with shrub encroachment.

We found that the concentrations of SOC, TN, and TP, as well as C:N, increased with shrub encroachment in clay soil. 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). 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.

We observed that SOC and TN increased in acidic soil after shrub encroachment, especially encroached by Leguminosae shrubs. This may be because that 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). 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).

### 4.4. The role of woody plant type

We found that compared to leguminous shrubs, the expansion of non-leguminous shrubs to grasslands increased soil P more, which may be because leguminous plants take up more P than non-leguminous plants as leguminous plants require more P to grow than non-leguminous plants (Li et al., 2011). 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.

### 4.5. Limitations and perspective

This study has several limitations. Soil microbes and enzymes were not considered, due to the lack of these data in literature. Thus, we cannot pay attention to the influence of microbial traits on soil nutrients cycling with shrub encroachment, which is a deficiency. The datasets were divided into different subgroups, which resulting in the sample size of each moderator decrease, such as arid climate, bottom soil layer, sandy clay texture. It would affect the statistical power and comprehensiveness of the meta-analysis.

When analyzing the influence of moderator factors on soil stoichiometry after shrub encroachment, variables controlling were not realized. The control variable is hard to examine in natural ecosystems, but would be a breakthrough point in future works. For most field experiments, the history of shrub encroachment was not reported. It can be showed from the results that the age of shrub encroachment is also an important factor, so more field experiments about shrub encroachment duration are needed to be conducted in the future. For better understanding and predicting how ecosystem functions respond to shrub expansion in grasslands, long-term studies from different ecosystems and climatic gradients are needed. In addition, we found that studies investigating the effects of shrub encroachment on soil stoichiometry are much less in comparison with that focusing on soil nutrient concentrations, more cross-site studies are required to assess the effects of shrub encroachment on soil stoichiometry.

## 5. 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 zones with semi-humid and extremely humid climate. The rate of increase in soil C:N decreases with the duration of shrub encroachment, suggesting a stabilization of soil C:N ratio in shrub-encroached grasslands. These findings provide fundamental knowledge for estimating the impacts of shrub encroachment on C and N cycling in grasslands, and help to better predict the effects of global change on grassland biogeochemical cycling and ecosystem services in a changing world.

## CRediT authorship contribution statement

**Zhong Du:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Methodology, Funding acquisition, Conceptualization. **Huan Zheng:** Writing – review & editing, Writing – original draft, Software. **Josep Penuelas:** Writing – review & editing. **Jordi Sardans:** Writing – review & editing. **Dongzhou Deng:** Writing – review & editing, Funding acquisition. **Xiaohu Cai:** Writing – review & editing. **Decai Gao:** Writing – review & editing, Writing – original draft, Software, Methodology. **Shirui Nie:** Writing – review & editing, Software. **Yanmin He:** Writing – review & editing, Software. **Xiaotao Lü:** Writing – review & editing, Writing – original draft, Methodology. **Mai-He Li:** Writing – review & editing, Writing – original draft, Methodology.

## Declaration of competing 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.

## Data availability

The authors are unable or have chosen not to specify which data has been used.

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## Appendix A. Supplementary data

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