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Intensification of no-till agricultural systems: An opportunity for carbon sequestration

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Abstract

The "4 per 1,000" initiative was launched at the 21st Conference of the Parties (COP21) stimulating a long-standing debate on the potential of no-till (NT) to promote soil C sequestration. Previous reviews found little or no soil organic C (SOC) accrual in NT soils as compared with full inversion tillage when soils are sampled deeper than 30 cm. Here, we present the results of a global meta-analysis of studies assessing SOC and total N (TN) storage and dynamics in NT and tilled soils from the most important agricultural regions of the world. Overall, our results show that NT soils stored 6.7 \pm 1.9 Mg C ha⁻¹ and 1.1 \pm 0.4 Mg N ha⁻¹ more than tilled soils (0-to-100-cm depth) with an average of 16 yr of NT, in contrast with previous findings. However, C sequestration $(+4.7 \pm 1.9 \text{ Mg C ha}^{-1})$ in the 0-to-60-cm depth with an average of 11 yr of NT) depended on the association of NT with increased crop frequency and the inclusion of legumes cover crops. Single-cropping systems lack the necessary C inputs to offset SOC losses in the soil profile (below 30-cm depth). However, double-cropping systems decreased soil TN that may constrain future C sequestration. The use of legumes alleviated TN loss and supported soil C sequestration. Briefly, our findings indicate that NT can avoid SOC losses from tilled soils, partially offsetting CO₂ emissions from agriculture. Moreover, NT with agricultural intensification can promote soil C sequestration, thus contributing to soil quality, food security, and adaptation to climate change.

INTRODUCTION 1

Soils have lost up to 133 Pg C over the past 12,000 yr due to the appropriation of lands under natural vegetation for agriculture (Sanderman et al., 2017). Soil organic C (SOC) losses

Abbreviations: AT, alternative tillage practices; CA, conservation agriculture; CP, chisel plow; DP, disk plow; MAP, mean annual precipitation; MAT, mean annual temperature; MP, moldboard plow; NT, no-till; SOC, soil organic carbon; TN, total nitrogen.

increased dramatically in the last 200 yr after further expansion of agriculture area under intensive tillage, decreasing original SOC stocks by 26 and 16% in the respective 0-to-30- and 0-to-100-cm soil layers (Sanderman et al., 2017). Soil organic C losses decrease soil quality threatening food security and contribute to climate change by increasing atmospheric CO_2 (Lal, 2010).

Conservation agriculture (CA; i.e., minimal soil disturbance, permanent soil cover, and crop rotation) is a major initiative promoted by FAO-UN and other institutions to recover

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SOC and provide climate change adaptation and mitigation (Jat et al., 2014). No-till (NT) agriculture is the basic component of CA by addressing at least two of its three principles (i.e., planting through soil cover with minimum soil disturbance; Nunes et al., 2020). Several studies have already shown that NT significantly increases SOC at the soil surface in a wide range of soils, climates, and cropping systems (Ogle et al., 2005; West & Post, 2002). In this sense, the "4 per 1,000" initiative was launched at the 21st Conference of the Parties (COP21) promoting CA and other agricultural practices to provide soil C sequestration as an alternative to mitigate atmospheric CO₂ (Minasny et al., 2017).

However, the "4 per 1,000" initiative has been highly debated as several studies pondered that an annual increase of SOC at 0.4% yr⁻¹ over a sufficiently long timeframe to significantly mitigate climate change is unattainable (Amelung et al., 2020; Baveye, 2021; de Vries, 2017; van Groenigen et al., 2017; VandenBygaart, 2017; White et al., 2017). Moreover, the capacity of NT soils to promote C sequestration has been also debated as recent reviews have indicated little or no differences in SOC storage between tilled and NT when soils are sampled deeper in the profile (>30 cm; Angers & Eriksen-Hamel, 2008; Luo et al., 2010). Other studies have suggested that SOC losses in deeper layers of both tilled and NT soils may offset any C accrual in the surface, resulting in a net C loss rather than sequestration (Olson et al., 2014; Stewart et al., 2017). These results were attributed to the redistribution of SOC in the soil profile with tillage (Angers & Eriksen-Hamel, 2008; Luo et al., 2010), large yield gap (Amelung et al., 2020; Ogle et al., 2012; Pittelkow et al., 2014; Stewart et al., 2017), and the lack of root C inputs to deeper soil layers (Baker et al., 2007; Dietzel et al., 2017; Wuaden et al., 2020), thus impairing SOC stocks and C sequestration throughout the profile of NT soils.

No-till experiments are relatively recent (<50 yr) and are often designed to replicate regional agricultural practices with limited N input and low crop frequency and diversity to promote C inputs levels conductive of significant soil C sequestration (Bayer, Martin-Neto, et al., 2006; Boddey et al., 2010; Corbeels et al., 2016; Follett et al., 2013; Martínez et al., 2016; Olson et al., 2013; Poffenbarger et al., 2017; Poirier et al., 2009; Stewart et al., 2017). However, recent studies show that the association of NT with best crop management practices (i.e., crop rotation, use of legume cover crops, intensification of cropping systems, use of organic amendments, and improved fertilizer management) could close the yield gap and increase C inputs to the soil, thus augmenting SOC stocks in NT soils up to the levels of natural soils and suggesting a greater potential for C sequestration than previously thought (Amelung et al., 2020; Cook & Trlica, 2016; de Oliveira Ferreira et al., 2016, 2018, 2020; Gonçalves et al., 2019; Grassini & Cassman, 2012; Grassini et al., 2015; Hok et al., 2015; Nicoloso et al., 2020).

Core Ideas

- Here, we present a meta-analysis on deep SOC and TN storage and dynamics in tilled and NT soils.
- No-till soils stored more SOC and were more conservative of TN than tilled soils.
- Differences in SOC stocks between tilled and NT soils increased with temperature.
- Carbon sequestration in NT soils increased with intensification of cropping systems.
- The use of legumes cover crops sustained TN stocks and promoted soil C sequestration under NT.

Nonetheless, previous meta-analysis comparing SOC stocks in NT and tilled soils focused on temperate agroecosystems with full inversion tillage (moldboard plow) and low crop frequency and diversity (Angers & Eriksen-Hamel, 2008; Luo et al., 2010). In fact, >85% of experiments included in these reviews were carried out in cold climate regions with one crop per year and occasional fallows. Moreover, both studies based their conclusions on the comparison of paired plots from long-term experiments with no pretreatment baselines, thus failing to account for temporal changes in SOC stocks that are likely to occur in both tilled and NT soils (Olson et al., 2014). All these aspects could bias the interpretation and extrapolation of their results for a global perspective of the potential of NT soils to deliver C sequestration, especially for warmer climate agricultural regions with better opportunities for the intensification of cropping systems.

Here, we reexamine the potential of NT to promote C sequestration and increase SOC storage throughout the soil profile (0-100 m) in comparison with tilled soils. We performed a global meta-analysis using the largest database so far on this topic including studies from the most important agricultural regions across the major temperature domains of the world (tropical, subtropical, and temperate). We also assessed total N (TN) storage and dynamics in both tilled and NT soils because of the constrained flexibility of their stoichiometry that regulates soil C sequestration (van Groenigen et al., 2017; Zaehle, 2013). Studies were screened for those comparing paired tilled and NT plots at a given sampling time and for those assessing temporal changes of tilled or NT soils in comparison with their pretreatment baselines (Olson et al., 2014). We also distinguished tilled soils in categorical groups according to the primary tillage type performed with moldboard plow (MP), disk plow (DP), chisel plow (CP), and other alternative tillage practices (AT). The impact the intensification of agricultural systems was also assessed by discriminating cropping systems according to frequency (single or double crop) and use of legumes (no legumes or with legumes). We hypothesized that (a) NT soils have greater SOC and TN stocks than tilled soils, especially in warm climate agricultural regions, and (b) the intensification of cropping systems increases the potential for C sequestration in NT soils.

2 | MATERIALS AND METHODS

2.1 | Data collection

We searched the Google Scholar, Web of Science, and Scopus databases for peer-reviewed articles, theses, and dissertations assessing SOC and/or TN storage in tilled and NT soils. The search resulted in 200 studies published until September 2017. We selected only 142 studies that directly reported or provided the information required for the calculation of SOC and TN stocks throughout the soil profile (that is, soil mass or soil bulk density and C and N contents in a given soil layer). Soil organic C and TN stocks were calculated for all soil layers in each treatment reported in the selected studies (Ellert & Bettany, 1995). We then adjusted the SOC and TN stocks measured in each treatment to equivalent soil masses (ESM) using an Excel add-in cubic spline macro function (SRS1 Software) that consisted of a piecewise series of cubic polynomial curves to calculate SOC stocks in cumulative soil masses (Wendt & Hauser, 2013).

The selected studies often reported different sampling depth intervals. Thus, in order to maximize the number of comparisons in this meta-analysis, we normalized the SOC and TN stocks in each study to the soil masses equivalent to the 0-to-5-, 5-to-15-, 15-to-30-, 30-to-60-, and 60-to-100-cm soil layers using the same procedure with cubic spline functions (Wendt & Hauser, 2013). For that, studies should have reported soil SOC or TN stocks from at least three depth intervals. For example, if a study reported results for the 0-to-10-, 10-to-20-, and 20-to-40-cm depths, we used the cubic spline functions to normalize soil SOC and TN stocks for the 0-to-5-, 5-to-15-, and 15-to-30-cm soil layers. Others studies reporting results only for the 0-to-80-cm soil layer, for example, were discarded.

The studies were further screened for those (a) comparing paired tilled and NT plots at the same sampling time, or (b) assessing temporal changes on tilled or NT soils in comparison with a pretreatment baseline from the same plot. We only selected those studies that had similar soil type and management practices in both treatments (that is, residue management, mineral or organic N fertilization, crop number [diversity] and frequency [number of crops per year], and legume use frequency in the cropping system). We discarded studies where tillage treatments were applied for <5 yr (Angers & Eriksen-Hamel, 2008; Virto et al., 2012). We also discarded studies or treatments where crop residue were removed, burned, or grazed (5% of the database), based on the criteria that NT agriculture requires permanent soil cover. Thus, only studies where crop residues were maintained or returned to the soil surface were included in our meta-analysis. Additionally, we included only the data from the last sampling in experiments with repeated measures and removed duplicate results published in different articles. This procedure narrowed our selection to 121 studies with a total of 266, 254, 218, 155, and 60 comparisons of SOC stocks in the 0-to-5-, 5-to-15-, 15-to-30-, 30-to-60-, and 60-to-100-cm soil layers of paired tilled and NT plots, respectively. Fewer studies (50) also reported TN stocks in paired NT and tilled soils, thus yielding 114, 110, 97, 76, and 34 comparisons in the same layers, respectively.

The same procedure was used to identify studies reporting temporal changes in SOC and TN stocks in tilled and NT soils in comparison with their pretreatment baselines. Since these studies reported a pretreatment baseline, we did not excluded studies based on the duration of tillage treatments. We thus selected 33 studies with 118, 118, 115, 68, and 34 comparisons for SOC stocks, and another 15 studies with 62, 62, 62, 45, and 29 comparisons for TN stocks in the same 0-to-5-, 5-to-15-, 15-to-30-, 30-to-60-, and 60-to-100-cm soil layers, respectively. Other information retrieved from the selected studies included location (latitude and longitude), mean annual temperature (MAT), mean annual precipitation (MAP), soil order (Soil Survey Staff, 2014), soil texture, soil clay content, tillage practice, tillage depth, and duration of the experiments. The MAT and MAP were estimated using national weather databases for studies not reporting weather data. Unfortunately, very few studies reported clay, silt, or sand contents in the correspondent soil depths where SOC and TN stocks were measured. Most studies reported only soil textural class (116 of 121 studies), and fewer reported clay content (89 of 121 studies) although frequently not stating sampling depth and only referring to a topsoil or plow layer. Thus, we were not able to investigate the effects of soil texture regulating SOC and TN storage in our metaanalysis.

Although a NT treatment was included in all studies, soil tillage practices varied significantly. We thus grouped tillage practices in categorical variables considering the equipment used to perform the primary tillage operation, with (a) MP, (b) DP, (c) CP, and (d) AT. Alternative tillage grouped studies using other tillage practices such as strip tillage, rotary tillage, sweep plow, ridge tillage, stubble-mulch tillage, shallow disking, or reduced frequency tillage. Crop frequency (single or double crop) and the use of legumes in the crop rotation (with or without) were also treated as categorical variables. Single crop indicated that one or fewer crops were grown in the experimental plot per year, and double crop indicated that two or more crops were grown in the experimental plot per year, on the average of the duration of the study. The complete

database used in this meta-analysis is included as supplemental material (Supplemental File 2).

2.2 | Data analysis

We extracted the mean and SD of SOC and TN stocks (Mg ha⁻¹) in each normalized sampling layer, and the sample size (*n*) from individual observations of NT and tilled soils within the selected studies. Where only SEs were given, SE was converted to SD, with SD = $SE\sqrt{n}$, where SD could not be determined, we assigned the SD as 1/10 of the mean (Luo et al., 2006). Tilled soils were used as the control treatment for the comparison with paired NT soils and the pretreatment baselines were used as the control treatment baselines were used as the control treatment to assess temporal changes on soil C and N stocks in both tilled and NT soils.

The standardized effect size (*E*) was calculated for the comparison of SOC and TN stocks between treatments in each sampling layer from all studies using the Hedges' *d*, with $d = [(\bar{x}_t - \bar{x}_c)/\sigma]J$, where \bar{x}_t and \bar{x}_c are the mean SOC and TN stocks of the tested and control treatments, respectively, σ is the pooled SD as calculated with $\sigma = \sqrt{[(n_t - 1)SD_t^2 + (n_c - 1)SD_c^2]/(n_t + n_c - 2)}$, and *J* accounts for unequal sampling variation among treatments as calculated with $J = 1 - 3/[4(n_t + n_c - 2) - 1]$. The variance of Hedges' *d* was found with $v_d = (n_t + n_c)/n_t n_c + d^2/[2(n_t + n_c)]$. The overall cumulative effect size (\bar{E}) was calculated using a random-effects model meta-analysis, with $\bar{E} = \sum_{i=1}^n w_i E_i / \sum_{i=1}^n w_i$, where *n* is the number of comparisons, E_i is the effect size for the *i*th comparison, and w_i is the weight of the *i*th comparison.

Similarly, the cumulative effect size for categorical groups (\bar{E}_j) was calculated with $\bar{E}_j = \sum_{i=1}^{k_j} w_{ij} E_{ij} / \sum_{i=1}^{k_j} w_{ij}$, where k_j is the number of comparisons in the *j*th group, and w_{ij} and E_{ij} are the weight and effect size for the *i*th comparisons in the *j*th group. The weight of individual comparisons $(w_i \text{ or } w_{ij})$ was reciprocal to its sample variance, calculated with $w_i = 1/[v_{d(i)} + \sigma_{\text{pooled}}^2]$ or $w_{ij} = 1/[v_{d(ij)} + \sigma_{\text{pooled}}^2]$, where $v_{d(i)}$ or $v_{d(ij)}$ is the variance of Hedges' *d* for the *i*th comparison or *i*th comparison in the *j*th group, and σ_{pooled}^2 is the betweenstudy variance. The σ_{pooled}^2 of \bar{E} was calculated with $\sigma_{\text{pooled}}^2 = [Q_{\text{T}} - (n-1)]/(\sum_{i=1}^n w_i - \sum_{i=1}^n w_i^2/\sum_{i=1}^n w_i)$, where *n* is the number of comparison, w_i is the fixed-effects model weight for the *i*th comparison calculated with $w_i = 1/v_{d(i)}$ and Q_{T} is the total heterogeneity for the sample of studies calculated with $Q_{\text{T}} = \sum_{i=1}^n w_i (E_i - \bar{E})^2$.

The σ_{pooled}^2 of \bar{E}_j was calculated with $\sigma_{\text{pooled}}^2 = \frac{Q_{\text{E}} - (n-m)}{\sum\limits_{i=1}^{m} (\sum\limits_{i=1}^{n} w_{ij} - \sum\limits_{i=1}^{k_j} w_{ij}^2 / \sum\limits_{i=1}^{k_j} w_{ij})}$

where *n* is the total number of comparisons, *m* is the number of groups, k_i is the number of comparisons in the *j*th group, w_{ii}

is the fixed-effects model weight for the *i*th comparison in the *j*th group calculated with $w_{ij} = 1/v_{d(ij)}$ and $Q_{\rm E}$ is the residual error heterogeneity for the sample of studies calculated with $Q_{\rm E} = \sum_{j=1}^{m} \sum_{i=1}^{k_j} w_{ij} (E_{ij} - \bar{E}_j)^2$. The SD of \bar{E} was calculated with ${\rm SD}_{\bar{E}} = \sqrt{1/\sum_{i=1}^{n} w_i}$, and the 95% confidence interval (CI) around \bar{E} was found with CI = $\bar{E} \pm t_{\alpha/2(n-1)} \times {\rm SD}_{\bar{E}}$, where *t* is the two-tailed critical value from the Student's *t* distribution ($\alpha = .05$). Similarly, the SD of \bar{E}_j was calculated with ${\rm SD}_{\bar{E}_j} = \sqrt{1/\sum_{i=1}^{k_j} w_{ij}}$. and the 95% confidence interval (CI) around \bar{E}_j was found with CI = $\bar{E}_j \pm t_{\alpha/2(n-1)} \times {\rm SD}_{\bar{E}_j}$ at $\alpha = .05$.

We used bootstrap resampling with 999 interactions to correct the calculated 95% CI for bias caused by small sample sizes or differences in data distribution among treatments, thus generating a 95% bootstrap CI. Both \bar{E} and \bar{E}_j were considered to be significant if its 95% bootstrap CI does not bracket zero (P < .05). We also assessed the heterogeneity between categorical groups by using a randomization test with 999 interactions. Differences between groups were considered significant when randomization tests yielded P values <.05.

For better interpretation, results were unstandardized and expressed in the original units as mean difference (MD) of soil carbon and nitrogen stocks by multiplying \overline{E} and \overline{E}_i by the average pooled SD. Although some studies have also reported (or we were able to calculate from their data) SOC and TN stocks for the cumulative 0-to-100-cm soil layer, we rather calculated a cumulative MD of soil SOC and TN stocks by adding the results of the stratified soil layers (Luo et al., 2010). The cumulative MD and their 95% bootstrap CI for the 0to-100-cm soil layer was found with $MD_c = \sum_{i=1}^{l} MD_i$ and $CI_c = \sqrt{\sum_{i=1}^{l} CI_i^2}$, where *l* is the number of soil layers, and MD; and CI; are the MD and its 95% bootstrap CI for the *i*th soil layer. We adopted this procedure because only a limited number of comparisons were performed for the whole 0-to-100-cm layer in relation to the number of comparisons made for stratified soil layers. Thus, our procedure was more representative of the MD of SOC and TN stocks by considering a larger number of comparisons within each soil layer.

We used the data from studies reporting SOC and TN stocks for the cumulative 0-to-30-, 0-to-60-, and 0-to-100-cm soil layers of paired NT and tilled soils to perform a continuous random-effects model meta-analysis assessing the effects of independent variables on the standardized effect sizes (*E*) of individual studies. We thus performed 266, 163, and 67 comparisons of C stocks and 111, 80, and 37 comparisons of N stocks in the cumulative 0-to-30-, 0-to-60-, and 0-to-100-cm soil layers, respectively. The relationship between *E* and the independent variables (MAT, MAP, and duration of the experiments) was determined using weighted least squares regressions with $E_i = b_0 + b_1 X_i + \varepsilon$, where E_i and X_i are the effect size and independent variable of the *i*th comparison, b_0 and

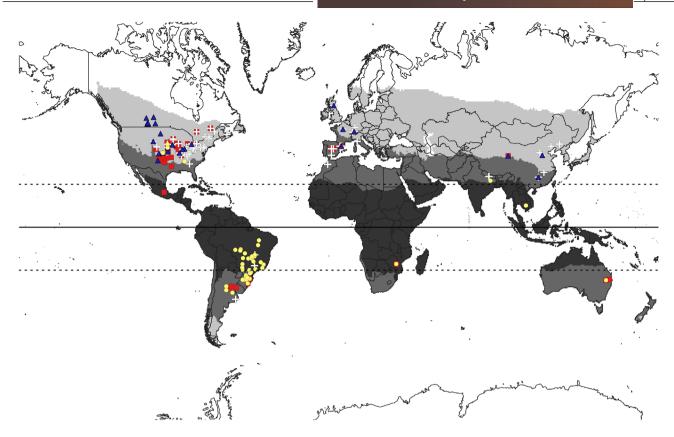


FIGURE 1 Location of the selected studies comparing moldboard plow (white crosses), disk plow (yellow circles), chisel plow (red squares), and alternative tillage (blue triangles) with no-tillage (all locations) in temperate (light gray), subtropical (gray), and tropical (dark gray) climate regions. The horizontal continuous line indicates the equator and the dotted lines indicates the tropics in the north and south hemispheres

 b_1 are the intercept and slope of the regression, and ε is the error.

We found the slope of the regression with

$$b_1 = \frac{\sum_{i=1}^n w_i X_i E_i - (\sum_{i=1}^n w_i X_i \sum_{i=1}^n w_i E_i) / \sum_{i=1}^n w_i}{\sum_{i=1}^n w_i X_i^2 - (\sum_{i=1}^n w_i X_i)^2 / \sum_{i=1}^n w_i}$$

The intercept of the regression was found with $b_0 = \sum_{i=1}^{n} w_i E_i - b_1 \sum_{i=1}^{n} w_i X_i$

The SEs of b_0 and b_1 were then calculated with $SE_{b_0} = 1/\sqrt{\sum_{i=1}^n w_i} - (\sum_{i=1}^n w_i X_i)^2 / \sum_{i=1}^n w_i X_i^2}$ and $SE_{b_1} = 1/\sqrt{\sum_{i=1}^n w_i X_i^2} - (\sum_{i=1}^n w_i X_i)^2 / \sum_{i=1}^n w_i}$, respectively. The weight of individual comparisons (w_i) was calculated as described above for random-effects models. However, the

 σ_{pooled}^2 for the continuous model was calculated with

$$\sigma_{\text{pooled}}^{2} = \frac{Q_{\text{E}} - (n-2)}{\sum_{i=1}^{n} w_{i} - \sum_{i=1}^{n} w_{i}^{2} \left[\frac{\sum_{i=1}^{n} w_{i} X_{i}^{2} - 2X_{i} \sum_{i=1}^{n} w_{i} X_{i} + X_{i}^{2} \sum_{i=1}^{n} w_{i}}{\sum_{i=1}^{n} w_{i} \sum_{i=1}^{n} w_{i} \sum_{i=1}^{n} w_{i} X_{i}^{2} - \left(\sum_{i=1}^{n} w_{i} X_{i}\right)^{2}} \right]}$$

The $Q_{\rm E}$ was calculated as already described.

We tested the significance of the slope with $Z = b_1/SE_{b_1}$ and comparing the Z score (Z) to a normal distribution. We calculated the amount of heterogeneity as explained by the regression model (Q_M) with $Q_M = b_1^2/SE_{b_1}^2$. The significance of Q_M was tested against a X^2 distribution with 1 df. Either a significant Z score or Q_M indicated that the independent variable explained a significant portion of the variation in effect sizes (P < .05). All statistical analysis were performed with MetaWin (version 2.1) (Rosenberg et al., 1999).

3 | **RESULTS AND DISCUSSION**

3.1 | SOC and TN stocks in paired NT and tilled plots

3.1.1 | Effect of soil tillage practices

Our meta-analysis summarized studies comparing different soil tillage and crop management practices covering six continents (Figure 1) with a wide range of soil types (8 of the 12 soil orders according to the USDA soil taxonomy [Soil Survey Staff, 2014]) and climates (MAT ranged from 0.7 to 28 °C and MAP ranged from 2.68 to 20.61 cm yr⁻¹). We were

able to perform 953 comparisons of SOC stocks and 431 comparisons of TN stocks in different soil layers of paired tilled and NT plots. These numbers of comparisons were four times higher than previous assessments concerning SOC and were unprecedented for TN (Angers & Eriksen-Hamel, 2008; Luo et al., 2010).

Overall, SOC stocks were greater in NT than in tilled soils, notably in the upper 0-to-5- and 5-to-15-cm soil layers (Figure 2a). The MD of SOC stocks in the topsoil layer (0–5 cm) followed a hierarchy according to soil disturbance: NT > AT > CP > DP = MP. Tillage practices that were grouped as "alternative tillage" represented the least soil disturbance and were performed at a shallower depth (13 \pm 7 cm, mean \pm SD) than other tillage groups (19–23 cm). Thus, differences in SOC between NT and AT were limited to the surface layer (0–5 cm).

Moldboard plow represented the greatest soil disturbance by fully inverting soil layers and reallocating fresh crop residues to the bottom of the plow layer $(23 \pm 5 \text{ cm})$, thus increasing SOC storage and stabilization in relation to NT in the 15-to-30-cm soil layer as reported elsewhere (Angers & Eriksen-Hamel, 2008; Luo et al., 2010). We did not find such inversion for the other tillage practices. Moreover, no differences on SOC stocks were noticed in deeper soil layers (30– 60 and 60–100 cm) on the comparison of tilled and NT soils, regardless of the tillage practice used in tilled soils.

No-till soils also had greater TN stocks than tilled soils but only at the surface soil layers (0–5 and 5–15 cm) after the increase of SOC observed in the same soil layers (Figures 2a and 2b). Increased SOC and TN stocks in response to C and N inputs and minimum soil disturbance were widely reported in previous studies from both temperate and tropical agroecosystems (de Campos et al., 2011; Nicoloso et al., 2018; Virto et al., 2012). Nonetheless, differences among soil tillage practices were noticed only in the topsoil layer (0–5 cm) where TN stocks followed a hierarchy that was similar to observed for SOC stocks: NT > AT = CP = MP > DP. No differences on TN stocks were observed between tilled and NT soils for any of the other soil layers assessed in this study (15–100 cm), regardless of the tillage practices used in tilled soils.

Mean differences in SOC and TN in each soil layer were then integrated to the cumulative 0-to-100-cm layer (Figures 3a and 3b). Overall, NT soils had greater SOC and TN stocks than tilled soils ($\pm 6.7 \pm 1.9$ Mg C ha⁻¹ and $\pm 1.1 \pm 0.4$ Mg N ha⁻¹, respectively). The average duration of NT was 17 and 16 yr in the studies comparing SOC and TN stocks, respectively, between NT and tilled soils. Thus, MD of SOC and TN stocks between NT and tilled soils increased by 0.40 \pm 0.11 Mg C ha⁻¹ yr⁻¹ and 0.07 \pm 0.02 Mg N ha⁻¹ yr⁻¹, respectively.

Mean differences of SOC were also significant for the comparison of NT with DP, CP, and MP soils, ranging from +6.3 \pm 1.9 to +8.6 \pm 3.7 Mg C ha⁻¹ or from 0.37 \pm 0.11 to 0.50 \pm 0.33 Mg C ha⁻¹ yr⁻¹. Thus, the greater SOC storage in the

15-to-30-cm soil layer of MP soils was not enough to offset increased SOC stocks in the upper layers of NT soils in the comparison of NT and MP soils, as reported in a previous meta-analysis (Luo et al., 2010). Moreover, the MD between NT and MP soils (+8.6 \pm 3.7 Mg C ha⁻¹) measured in our study was 75% higher than that observed by Angers and Eriksen-Hamel (2008) for a similar duration of the analyzed studies (+4.9 Mg C ha⁻¹ after 16 yr of NT). However, SOC stocks did not differed between NT and AT soils (+2.5 \pm 3.5 Mg C ha⁻¹), thus suggesting that the limited soil disturbance promoted by AT were more conservative of SOC than the other tillage practices used in tilled soils. Nonetheless, TN stocks were greater under NT in comparison with any of the tested tillage groups, ranging from $+0.7 \pm 0.4$ to $+1.6 \pm 0.5$ Mg N ha⁻¹ or from 0.04 ± 0.02 to 0.10 ± 0.03 Mg C ha⁻¹ yr⁻¹. Thus, the MD observed between NT and tilled soils at the soil surface were sustained for the cumulative 0to-100-cm soil layer.

3.1.2 | Effects of crop frequency and the use of legumes

We also tested differences in SOC and TN storage between NT and tilled soils by grouping studies according to cropping frequency and the use of legumes. These practices are complementary to NT according to the principles of CA (Jat et al., 2014). The intensification of cropping systems was reported to increase both SOC and TN stocks under NT in response to higher annual biomass production in comparison to single cropping or fallowing systems (Calegari et al., 2008; Hok et al., 2015). Previous studies also reported that SOC stocks increased similarly in both tilled and NT soils in response to C inputs (Bayer, Lovato, et al., 2006; Stewart et al., 2007), and therefore differences between NT and tilled soils would be expected to remain constant regardless of crop frequency. However, a previous meta-analysis reported higher SOC stocks in the 0-to-30-cm layer of NT than in tilled soils in response to increase on C inputs (Virto et al., 2012). This effect was also observed in our study where double cropping systems increased SOC and TN storage under NT in comparison with tilled soils, but only at soil surface layer (0-5 cm) (Figures 2c and 2d). For the cumulative 0-to-100-cm soil layer, crop frequency had no effect on the MD of SOC and TN stocks between NT and tilled soils (Figures 3a and 3b), in contrast with a previous review (Luo et al., 2010).

Mean differences of SOC stocks between NT and tilled soils were also not affected by the inclusion of legumes in the cropping system for any of the individual or cumulative soil layers (Figure 2e and 3a). However, the inclusion of legumes had a strong effect on the MD of TN stocks between NT and tilled soils. We did not observe any differences in TN stocks between NT and tilled soils when legumes were absent in the

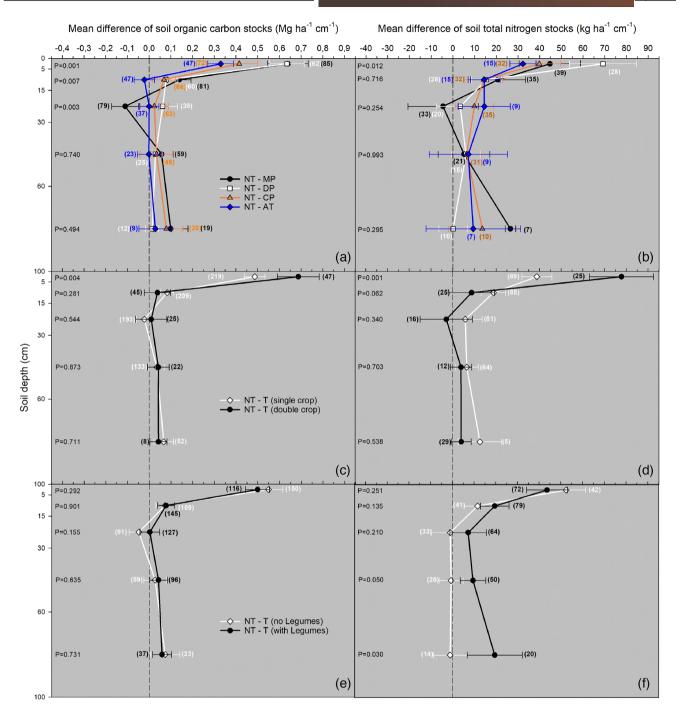


FIGURE 2 Mean differences of (a, c, e) soil organic C (SOC) and (b, d, f) total N (TN) stocks in the 0-to-5-, 5-to-15-, 15-to-30-, 30-to-60-, and 60-to-100-cm layers between no-till (NT) and tilled soils (dotted vertical lines) according to different (a, b) tillage practices, (c, d) crop frequency, and (e, f) use of legumes. Mean differences between no-till and tilled soils (NT – T) were grouped according to primary the tillage practices used in tilled soils (moldboard plow [MP], disk plow [DP], chisel plow [CP], and alternative tillage [AT]). Error bars show the 95% bootstrap confidence interval (CI). A positive mean difference indicates higher SOC or TN storage in NT soils in comparison with tilled soils, whereas a negative mean difference indicate higher SOC or TN storage in tilled soils. If the 95% bootstrap CI does not bracket zero, differences are considered significant (P < .05). *P* values indicate differences between treatments (tillage practices, crop frequency, and the use of legumes). Number of comparisons is given in parenthesis

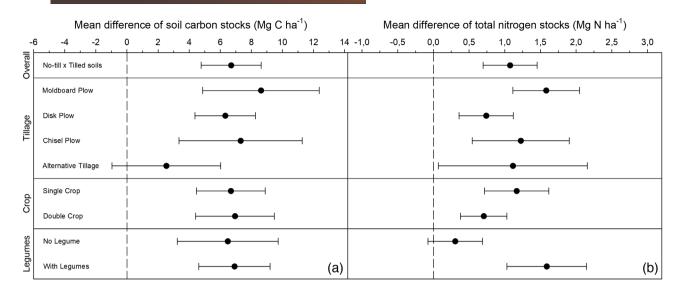


FIGURE 3 Mean differences of (a) soil organic C (SOC) and (b) total N (TN) stocks in the cumulative 0-to-100-cm layer between no-till (NT) and tilled (T) soils. Mean differences between no-till and tilled soils were also grouped according to primary tillage practices used in tilled soils (moldboard plow, disk plow, chisel plow, and alternative tillage practices), crop frequency (single or double crop) and use of legumes in the crop rotation (no legumes or with legumes). Error bars show the 95% bootstrap confidence interval (CI). A positive mean difference indicates higher SOC or TN storage in NT soils in comparison with tilled soils, whereas a negative mean difference indicates higher SOC or TN storage in tilled soils. If the 95% bootstrap CI does not bracket zero, differences are considered significant (P < .05)

cropping systems (+0.3 \pm 0.4 Mg N ha⁻¹ in the 0-to-100cm soil layer) (Figure 3b). However, NT soils had TN stocks +1.6 \pm 0.6 Mg N ha⁻¹ greater than tilled soils when legumes were included, as measured in the cumulative 0-to-100-cm soil layer. This result was mostly due to increased differences in TN stocks in the deeper soil layers (30–100 cm) with the use of legumes for the comparison of NT with tilled soils (Figure 2f).

Studies have shown higher N fixation rates for soybean and other legumes in NT than in tilled soils, with positive impacts on both SOC and TN stocks (Vieira et al., 2009; Zotarelli et al., 2012). In contrast, soil disruption was shown to increase SOC and moreover TN mineralization rates in both surface and deeper soil layers (Banegas et al., 2019; Hobley et al., 2018; Wuaden et al., 2020). Additionally, the use of legumes possibly prevented increased N scavenging by agricultural crops in deeper soil layers as compared with cropping systems lacking legumes with limiting N availability, thus increasing MD of TN stocks on the comparison of NT with tilled soils (Hobley et al., 2018).

3.1.3 | Effects of climate and duration of NT

Contrary to a previous assessment (Angers & Eriksen-Hamel, 2008) and in agreement with others (Luo et al., 2010; Virto et al., 2012), we did not find a correlation between the duration of NT and the MD of SOC stocks between NT and tilled soils (Table 1). Possibly because most of the changes of SOC stocks were reported to occur within 10 yr after the adoption

of NT (West & Post, 2002). Nonetheless, we found a positive correlation between soil TN stocks and the duration of NT for both 0-to-30- and 0-to-60-cm soil layers (*P* regression. = .047 and .016, respectively). In contrast with SOC, changes in TN stocks are known to be noticeable over a longer period. For instance, significant changes on TN stocks were still reported in the Broadbalk plots even after 116 yr of continuous winter wheat (*Triticum aestivum* L.) under MP (Glendining et al., 1996; Johnston & Powlson, 1994). Nonetheless, TN accumulation rates decreases with soil C/N ratio, whereas N losses increases proportionally with N inputs (Schipper & Sparling, 2011; Zaehle, 2013).

The MD of SOC and TN were positively correlated regardless of sampling layer (P < .001). Although N fertilization and the use of legumes had already been reported to increase SOC stocks in both tilled and NT soils (Poffenbarger et al., 2017; Stewart et al., 2017; Vieira et al., 2009), this result indicates that agricultural practices that favored a positive N balance also favored SOC storage in NT soils as compared with tilled soils (Zotarelli et al., 2012). Nonetheless, in contrast with that observed for SOC, the MD of TN stocks in the 0-to-100-cm layer between NT and tilled soils were negatively correlated with both MAT and MAP (slope = -0.05 ± 0.10 and -0.07 \pm 0.11, *P*-slope = .022 and .012, respectively). Reactive N losses (that is, NH₄ volatilization and losses of NO₃, N₂O, and other by-products of microbial processes) increase in NT soils in response to temperature, precipitation, and N inputs (Grave et al., 2018; Rochette et al., 2009). Thus, differences in TN stocks between tillage systems decreases with the increase in MAT and MAP.

Variable	Soil layer	Cmp.	Intercept	SE (I)	Slope	S.E. (S)	P-slope	P-reg.	
	cm	no.	Mg C ha ⁻¹		Mg	—Mg C ha ⁻¹ U ⁻¹ —		<i>P</i> value	
	MD SOC								
NTd (yr)	0–30	267	2.53	0.70	0.06	0.02	.928	.116	
	0–60	164	3.25	1.37	0.06	0.03	.748	.439	
	0–100	68	8.89	2.70	0.12	0.10	.725	.369	
MD TN (°C)	0–30	112	-2.01	0.77	3.62	0.17	.010	<.001	
	0–60	81	0.57	1.15	6.48	0.05	<.001	<.001	
	0–100	38	-7.49	1.96	2.59	0.44	<.001	<.001	
MAT (°C)	0–30	267	1.23	0.93	0.16	0.01	.995	.010	
	0–60	164	-0.45	1.86	0.40	0.01	.002	.008	
	0–100	68	14.03	3.72	-0.23	0.30	.144	.369	
MAP (cm)	0–30	267	3.24	0.84	0.02	0.04	.586	.756	
	0–60	164	3.45	1.83	0.07	0.07	.662	.680	
	0–100	68	11.68	3.09	-0.08	0.29	.305	.793	
					MD TN				
NTd (yr)	0–30	112	0.26	0.12	0.01	0.01	.974	.047	
	0–60	81	0.09	0.22	0.03	0.01	.999	.016	
	0–100	38	0.87	0.72	0.05	0.02	.907	.175	
MAT (°C)	0–30	112	0.41	0.16	0.00	0.01	.630	.731	
	0–60	81	0.20	0.26	0.03	0.01	.958	.127	
	0–100	38	2.48	0.84	-0.05	0.10	.022	.357	
MAP (cm)	0–30	112	0.45	0.17	0.00	0.01	.500	.943	
	0–60	81	0.59	0.26	0.00	0.02	.435	.909	
	0–100	38	2.33	0.64	-0.07	0.11	.012	.309	

TABLE 1 Relationship between duration of no-till (NTd), mean annual temperature (MAT), and mean annual precipitation (MAP) with the mean difference (MD) of soil organic C (SOC) and total N (TN) stocks between no-till and tilled soils

Note. Cmp., number of comparisons; S.E.(I) and S.E.(S), standard error of the intercept and slope; *P*-slope and *P*-reg., *P* value of the slope and regression; U, unit of the independent variable.

Mean annual temperature was positively correlated with the MD of SOC for the 0-to-30- and 0-to-60-cm layers of NT and tilled soils (*P* regression = .010 and .008, respectively). However, SOC was not correlated with MAP, regardless of soil depth. Although climate was known to regulate SOC (Delgado-Baquerizo et al., 2017), previous reviews also found no correlations between either temperature or precipitation with SOC on the comparison of NT and tilled soils (Luo et al., 2010; Virto et al., 2012). In contrast, our results indicate that differences between NT and tilled soils were negligible in colder climates, especially when soils were sampled deeper than 30 cm. For instance, the MD of SOC between tilled and NT soils calculated for the 0-to-60-cm soil layer ranged from -0.2 to 10.7 Mg C ha⁻¹ according to the MAT variation observed in our study (0.7–28 °C).

Spring tillage helps to increase soil temperature in cold and humid climate regions, providing better crop growth in comparison with NT soils (Licht & Al-Kaisi, 2005; Ogle et al., 2012; Pittelkow et al., 2014). Additionally, tillage decreases soil bulk density stimulating crop roots development in the soil profile (Baker et al., 2007). Both factors favor aboveground biomass production and root-derived C inputs for tilled soils, thus minimizing differences of SOC stocks as compared with NT soils from cold and humid regions. In contrast, residue retention at the surface of NT soils decreases soil temperature and increases water availability favoring crop growth in warmer and more arid agroecosystems (Pittelkow et al., 2014). Thus, MD of SOC between NT and tilled soils increases in warm climate regions where NT could promote higher C inputs to the soil and tillage operations favors SOC mineralization (Delgado-Baquerizo et al., 2017). Consequently, for every 1 °C increase in MAT, MD of SOC between NT and tilled soils augmented by 0.40 \pm 0.01 Mg C ha⁻¹ in 0-to-60-cm soil layer (*P*-slope = .002).

3.2 | SOC and TN dynamics NT and tilled soils

3.2.1 | Effect of soil tillage practices

Although both SOC and TN stocks were consistently higher in NT than in tilled soils, previous studies argued that the

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comparison of paired treatments was inadequate to assess C sequestration because the underlying assumption that the reference treatment (tilled soils) was at steady state often failed (Olson et al., 2014). Without a pretreatment baseline for comparison, differences in SOC stocks can result, for example, either from C sequestration in NT soils as well as from increased SOC losses in tilled soils. Although NT could offset CO₂ emission from agriculture by avoiding SOC losses from tilled soils, only C sequestration in NT soils could help to mitigate atmospheric CO₂ levels and improve soil quality (Olson et al., 2014). Thus, we assessed the potential of NT soils to promote C sequestration by performing a second meta-analysis identifying those studies from our database that also reported pretreatment baselines.

Unfortunately, the number of studies reporting data for the 60-to-100-cm soil layer was rather limited with only five experiments (one from Cambodia and four from the United States). Thus, although we present data from all individual soil layers (0–100 cm) in Figure 4, we integrated SOC and TN stocks only to the cumulative 0-to-60-cm soil layer (Figure 5), which had a better CI with a greater number of studies that were more representative of the entire database.

Both NT and tilled soils had significant SOC accrual in the 0-to-5- and 5-to-15-cm soil layers in comparison with their pretreatment baselines (Figure 4a). Previous studies have already reported significant C sequestration in the surface layers C-depleted soils in response to increased C inputs and N fertilization regardless of soil tillage system (Nicoloso et al., 2018, 2020; Poffenbarger et al., 2017; Stewart et al., 2017). Thus, SOC accumulation in the surface layers contributed up to 60% of the total C sequestration of 4.7 \pm 1.9 Mg C ha⁻¹ observed in the 0-to-60-cm soil layer after 11 yr of continuous NT (Figure 5a). Thus, the C sequestration rates observed in NT soils averaged 0.42 \pm 0.17 Mg C ha⁻¹ yr⁻¹. In contrast, SOC losses in the 30-to-60-cm layer of tilled soils completely offset SOC accumulation in the topsoil layers, resulting in SOC stocks that did not differ from the baseline in the cumulative 0-to-60-cm soil layer $(-0.2 \pm 1.8 \text{ Mg C ha}^{-1} \text{ after})$ 14 yr).

Previous studies have shown that SOC losses in deeper soils layers could result in a net loss of profile SOC in both tilled and NT soils (Olson et al., 2014; Stewart et al., 2017; Zotarelli et al., 2012). Authors have argued that both greater C inputs (Stewart et al., 2017) and a positive N balance (that is, fertilizer and legume inputs minus exportation through grain harvesting) (Zotarelli et al., 2012) are required to maintain profile SOC stocks even in NT soils. We found an accumulation of TN only in the 0-to-5-cm layer of NT soils (Figure 4b). However, both tilled and NT soils lost TN in the 30-to-60-cm layer, resulting in a total loss of -0.4 ± 0.3 and -0.3 ± 0.2 Mg N ha⁻¹ for the 0-to-60-cm soil layer, respectively, compared with their pretreatment baselines (Figure 5b). The average duration of studies reporting temporal changes on TN stocks was 7 yr for both NT and tilled soils.

3.2.2 | Effects of crop frequency and the use of legumes

Soil organic C stocks were positively affected by increasing crop frequency and the use of legumes in both tilled and NT soils. Regardless of soil tillage system, cropping systems with two or more crops per year (double crop) increased SOC up to a 30-cm depth in comparison with their pretreatment baseline, whereas the use of legumes favored SOC accrual throughout the measured soil profile in comparison with both pretreatment baseline and with treatments lacking the use of legumes (Figures 4c and 4e). Soils cultivated once per year or lacking legumes had accumulation of SOC in the 0-to-5-cm soil layer, but SOC losses observed at depth offset gains at surface soil layers.

When results were integrated to the 0-to-60-cm soil layer, SOC stocks in tilled soils did not differed from their pretreatment baseline regardless of the use of single $(-1.2 \pm 2.2 \text{ Mg} \text{ C} \text{ ha}^{-1})$ or double cropping systems $(+2.4 \pm 3.1 \text{ Mg} \text{ C} \text{ ha}^{-1})$. In addition, no significant changes on SOC were observed for tilled soils including legumes $(+2.6 \pm 3.2 \text{ Mg} \text{ C} \text{ ha}^{-1})$. However, the lack of legumes promoted significant SOC losses of $-2.3 \pm 2.1 \text{ Mg} \text{ C} \text{ ha}^{-1}$ in the cumulative 0-to-60-cm soil layer of tilled soils (Figure 5a). In contrast, SOC stocks in NT soils were significantly higher than their respective pretreatment baselines with either single crop $(+3.1 \pm 2.6 \text{ Mg} \text{ C} \text{ ha}^{-1})$ or double crop $(+7.3 \pm 2.7 \text{ Mg} \text{ C} \text{ ha}^{-1})$ and with the use of legumes $(+7.4 \pm 2.4 \text{ Mg} \text{ C} \text{ ha}^{-1})$. When legumes were absent, SOC stocks in NT soils did not differed from their pretreatment baselines $(+1.9 \pm 2.7 \text{ Mg} \text{ C} \text{ ha}^{-1})$.

Soil TN stocks remained stable at the surface soil layer (0–5 cm) but decreased significantly throughout the soil profile (5–60 cm) with the use of double cropping systems (Figure 4d). In contrast, single cropping systems showed accumulation of TN in the surface soil layers (0–15 cm) after the increase in SOC observed in the same soil layers (Figures 4c and 4d). This increase in TN stocks at the soil surface compensated TN losses observed at depth (30–60 cm). Thus, when TN stocks were integrated to the 0-to-60-cm layer, both NT and tilled soils using single cropping systems did not differed from their pretreatment baselines (-0.1 ± 0.3 and 0.2 ± 0.3 Mg N ha⁻¹, respectively). Nonetheless, double cropping systems showed significant losses of -0.7 ± 0.2 Mg N ha⁻¹ under NT, which were augmented to -1.3 ± 0.2 Mg N ha⁻¹ with soil disruption (Figure 5b).

Double cropping systems are widely used in subtropical and tropical climate regions where warmer temperatures allow two or more cultivations per year. The intensification of agricultural systems increases crop biomass production and C

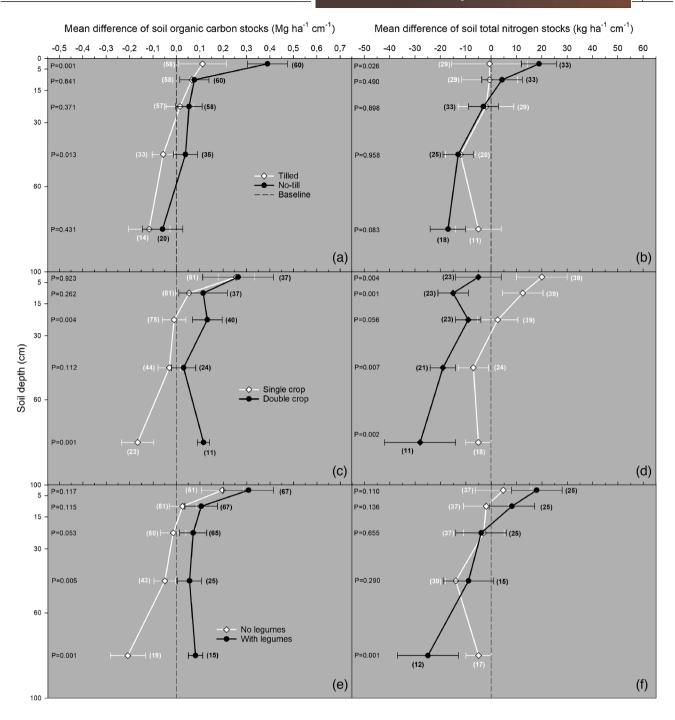


FIGURE 4 Mean differences of (a, c, e) soil organic C (SOC)) and (b, d, f) total N (TN) stocks in the 0-to-5-, 5-to-15-, 15-to-30-, 30-to-60-, and 60-to-100-cm layers due to the single effect of treatments with contrasting (a, b) soil tillage, (c, d) crop frequency, and (e, f) the use of legumes in comparison with their pretreatment baselines (dotted vertical line). Error bars show the 95% bootstrap confidence interval (CI). Treatments having a positive mean difference indicates SOC or TN accumulation, whereas a negative mean difference indicates SOC or TN loss in comparison with the pretreatment baselines. If the 95% bootstrap CI does not bracket zero, differences are considered significant (P < .05). P values indicate differences between treatments (soil tillage, crop frequency, and the use of legumes). Number of comparisons is given in parenthesis

inputs to the soil sustaining increased SOC stocks (Nicoloso et al., 2018, 2020; Poffenbarger et al., 2017; Stewart et al., 2017). Nonetheless, the use of double cropping systems could increase N scavenging by agricultural crops under limiting N availability (Hobley et al., 2018). Moreover, N losses are intensified in warmer climate regions, whereas a negative N

balance due to increased nutrient exportation with grain harvesting may have contributed to the losses of TN observed with increased crop frequency (Zotarelli et al., 2012). Thus, the increase of SOC and the concomitant decrease of TN stocks with the intensification of cropping systems could promote a decoupling of C/N cycles due to changes in C and N

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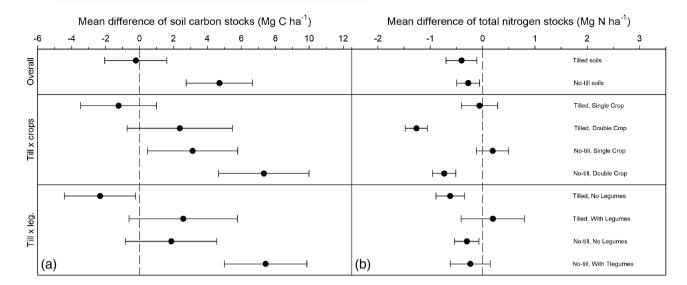


FIGURE 5 Mean differences of (a) soil organic C (SOC) and (b) total N (TN) stocks in the cumulative 0-to-60-cm layer of no-till (NT) and tilled (T) soils in comparison with their pretreatment baselines. Studies were also grouped according to crop frequency (single or double crop) and the use of legumes in the crop rotation (no legumes or with legumes). Error bars show the 95% bootstrap confidence interval (CI). Treatments having a positive mean difference indicates SOC or TN accumulation, whereas a negative mean difference indicates SOC or TN loss in comparison with the pretreatment baselines. If the 95% bootstrap CI does not bracket zero, differences are considered significant (P < .05)

inputs quality and quantity to the soil, soil disruption increasing SOC and TN mineralization rates, and increased N scavenging by agricultural crops (Banegas et al., 2019; Hobley et al., 2018; Wuaden et al., 2020).

Cropping systems lacking legumes also had significant TN losses in the 30-to-60-cm soil layers in comparison with their pretreatment baseline (Figure 4f). No differences were observed for other soil layers (0–30 cm). In contrast, legumes increased TN stocks at the soil surface (0–5 cm), whereas no differences were observed deeper in the soil profile. For the cumulative 0-to-60-cm soil layer, the lack of legumes decreased TN stocks in NT soils by -0.3 ± 0.2 Mg N ha⁻¹ (Figure 5b). Greater TN losses were observed in tilled soils lacking the use of legumes in the cropping system (-0.6 ± 0.3 Mg N ha⁻¹).

The use of legumes was found to offset TN losses in both tilled and NT soils ($+0.2 \pm 0.6$ and -0.2 ± 0.4 Mg N ha⁻¹, respectively), while supporting increased SOC in NT soils. Thus, our results suggest that both the intensification of agricultural systems and the use of legumes are required to provide necessary C and N inputs to support C sequestration and sustain TN stocks in NT soils (Poffenbarger et al., 2017; van Groenigen et al., 2017; Zaehle, 2013). However, the increasing frequency of soybeans [*Glycine max* (L.) Merr.] in rotation with maize (*Zea mays* L.) was already shown to limit SOC accumulation under NT in both temperate and tropical agroecosystems due to decreased C inputs in comparison with maize monocropping (de Oliveira Ferreira et al., 2016, 2018, 2020; Poffenbarger et al., 2017; Nicoloso et al., 2020). Thus, other species of legumes must be considered when designing

cropping systems to favor soil C sequestration, such as legume cover crops.

Double cropping systems including legume cover crops in rotation with grain cereals are often used in subtropical and tropical regions, providing increased C and N inputs and SOC accrual in NT soils (Amado et al., 2006; Boddey et al., 2010; Calegari et al., 2008; Hok et al., 2015; Vieira et al., 2009). Such cropping systems alternating grain cereals with legume cover crops could be a cost-effective alternative for soybean– maize rotation when designing climate-smart agroecosystems to promote soil C sequestration. Further efforts of farmer and scientists are necessary to adapt double-cropping systems with legume cover crops for temperate climate regions.

4 | CONCLUSIONS

Here, we show that SOC and TN balance in the soil profile is strongly dependent on the interactions of climate, crop frequency, and use of legumes regulating C and N inputs for both tilled and NT soils. Overall, NT soils stored more SOC and TN than intensively tilled soils, especially in warm climate agroecosystems that favored a positive TN balance with the use of legumes in the cropping system. Nonetheless, C sequestration in the whole soil profile was more likely when NT was associated with increased cropping frequency and the use of legumes, promoting greater C inputs and supporting TN stocks.

Our results also suggest that inventories regarding C sequestration in agricultural soils should be conducted at a

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regional scale considering changes of both SOC and TN stocks over time according to site-specific soil tillage practices and cropping systems. Moreover, scientists and farmers should focus on designing improved NT cropping systems in order to increase the potential of agricultural soils to promote C sequestration, especially for temperate climate regions, thus enhancing our efforts to improve soil health and provide food security. In this sense, agroecosystems should necessarily address the three principles of CA concerning minimum soil disturbance, intensification of agricultural systems, and further use of legume cover crops.

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AUTHOR CONTRIBUTIONS

Rodrigo S. Nicoloso: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing-original draft; Writing-review & editing. Charles W. Rice: Conceptualization; Funding acquisition; Project administration; Supervision; Writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

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