STRATIFICATION RATIOS IN A RAINFED MEDITERRANEAN VERTISOL IN WHEAT UNDER DIFFERENT TILLAGE, ROTATION AND N FERTILISATION RATES

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Abstract

Semiarid Mediterranean climatic conditions and intensive tillage systems accelerate soil organic matter losses. Therefore, assessing agricultural practices that enhance storage of soil organic matter is needed. Stratification of soil properties with soil depth, expressed as a ratio, could indicate soil quality under different soil management. We measured soil depth stratification ratio (0-5 / 10-30 cm and 0-5 / 30-50 cm) of soil organic C (SOC), total N, active carbon (AC), water soluble carbon (WSC), and soil enzymatic activities [dehydrogenase activity (DHA) and β-glucosidase activity (BGA)] of a Typic Haploxerert in southern Spain. The experimental design consisted of a split-split plot design with three replications and soil properties evaluated at the end of 22 years. Tillage systems included conventional tillage (CT) and no tillage (NT). Dryland, 2-year crop rotations were wheat (Triticum aestivum L.)-fallow (WF), wheat-chickpea (Cicer arietinum L.) (WC), wheat-faba bean (Vicia faba L.) (WFb), wheat-sunflower (Helianthus annuus L.) (WS), and continuous wheat (WW). Nitrogen fertiliser rates were 0, 50 and 150 kg N ha^{-1}. Stratification ratios of total N, WSC, AC, DHA, and BGA were most responsive to tillage systems; NT greater than CT. Stratification ratios of SOC, total N, WSC, AC and BGA were most responsive to crop rotation; WFb and WW greater than WF, WC, and WS. Stratification ratio of BGA was most responsive to N fertiliser rate; higher rates than no fertiliser. Stratification ratios of C and N fractions and enzymatic activities were responsive to choice of denominator used for ratio calculation. Tillage and crop rotation had more influence than N fertiliser rate in affecting stratification ratio of C and N fractions and enzymatic activities. Stratification ratio were relatively low (<2), perhaps due to the large shrinking and swelling characteristics of Vertisols. Stratification ratio of BGA was greater than of all other soil properties, suggesting that it might be a good indicator of soil quality under different soil management in Mediterranean

conditions, particularly with Vertisols. This study indicated the value of stratification ratios to detect improvement in soil organic matter fractions and enzymatic activities (i.e. indicators of soil quality) with adoption of improved conservation management approaches.

1. Introduction

Conservation tillage can alter nutrient availability compared with conventional tillage (CT) due to surface placement of residues rather than mixing of residues within the tilled soil zone (Franzluebbers and Hons, 1996). Tilled soils have a more uniform distribution of soil organic matter (SOM) with depth compared with a highly stratified distribution of SOM with no tillage (NT) (Franzluebbers, 2004). Plant residue placement is of importance to the depth distribution of SOM in the soil profile, because plant residues contribute greatly to subsequent SOM formation. Therefore, stratification of SOM fractions has been suggested as an indicator of soil quality in different agroecological zones, because surface SOM is essential to erosion control, conservation of nutrients, water infiltration, and other important soil functions (Franzluebbers, 2002). In general, and irrespective of soil and climatic conditions, a high stratification ratio (relation between surface and deeper layer concentrations of SOM) would indicate good soil quality, as ratios (>2) are not frequently found in degraded soils (Franzluebbers, 2002).

Effects of tillage on SOM fractions are dependent on the interplay of soil texture, climatic conditions, cropping systems, and timing, frequency, and depth of tillage (Six et al., 2002; Franzluebbers, 2004; Cookson et al., 2008). In general, SOM concentration is lower in

coarse-textured than in fine-textured soils, primarily due to a lack of physical protection of organic matter in sandy soils (Hassink, 1995; Feller and Beare, 1997). Hot and wet climates present a condition more suitable for rapid decomposition of SOM compared with cold and dry climates (Franzluebbers, 2002; Roldán et al., 2005). Changes in SOM and soil structure under conservation tillage systems can also substantially affect soil microbial biomass and its activity (Doran, 1980; Rabary et al., 2008; Melero et al., 2009). Several studies have reported that soil biological properties are greater under NT than under CT (Eivazi et al., 2003; Melero et al., 2009; Qin et al., 2010).

Under hot, semiarid climatic conditions (where soils usually have low SOM contents), determination of SOC alone might not be the best indicator of soil quality, because changes may be slow with high decomposition of SOM. In such conditions, more active fractions of SOM may be more sensitive to early changes in soil management than SOC (Haynes, 2005). Water-soluble C (WSC) is considered the most dynamic C fraction in soil, being a readily available substrate for microbial activity (McGill et al., 1986). Active carbon (AC) is an indicator of microbial activity and may be more sensitive to soil management than SOC (Weil and Magdoff, 2004).

Some enzymatic activities have been also considered as early indicators of soil change caused by different management (Paz-Ferreiro et al., 2009). Dehydrogenase activity (DHA) is an indicator of the microbial redox system and reflects potential oxidative activity of soil (Trevors, 1984); thus may be a good indirect measurement of soil biological activity. β-glucosidase activity (BGA) is involved in the C cycle, hydrolyzing β-D-glucoside into glucose, which is an important energy source for soil microorganisms (Eivazi and Tabatabai, 1988). Therefore, under semi-arid conditions, calculation of stratification ratio of SOC, as well as of other SOM fractions and other biochemical

properties closely related to SOM transformations and nutrient cycling, may be practically revealing to how soil management affects soil quality.

Franzluebbers (2002) suggested that more research on stratification ratio of various SOM fractions was needed to test the applicability of using stratification ratio as a soil quality indicator in different agroecological zones. Little information is available in the literature describing the interaction of tillage, crop rotation, and N fertilisation rate on depth stratification of SOM fractions. Therefore, our objective was to assess the long-term impact of conventional and conservation management on stratification ratio of various SOM fractions and enzymatic activities in a dryland Vertisol in southern Spain. We also wanted to test the relative importance of tillage, crop rotation, N fertiliser rate, and soil depth increments on stratification ratio of SOM fractions and enzymatic activities. We hypothesized that stratification ratio would be greater (i) under NT than under CT due to influences of crop residue placement on SOM fractions, (ii) under more intensive crop rotations than under wheat-fallow due to importance of crop residues on SOM fractions, (iii) under high N fertilisation doses than without N fertilisation due to production of greater amount of crop residues and its influence on SOM fractions, and (iv) with depth ratio of 0-5 / 30-50 cm than with depth ratio of 0-5 / 10-30 cm due to avoidance of tillage-induced SOM enhancement at lower levels of the plow layer (e.g. 20-30 cm).

2. Material and methods

2.1. Experimental site and management of tillage systems

A long-term field experiment was initiated in 1986 at Córdoba, Spain (37°46' N, 4°31' W, 280 m above sea level) on a Vertisol (Typic Haploxererts) with water holding capacity of ~350 mm m^{-1}, clay content of 700 g kg^{-1}, and sand content of 135 g kg^{-1}, where rainfed cropping is the standard practice in the Mediterranean region. This soil is characterized

by an AC profile with 30 cm of A horizont depth. Temperature and rainfall from 2002 to 2008 experimental period are shown in Figure 1. The experiment was a randomized complete block with a split-split-plot design and three blocks. Main plots were tillage system (CT and NT). Subplots were crop rotation, with four different 2-year rotations [wheat (*Triticum aestivum* L.)-fallow (WF), wheat-chickpea (*Cicer arietinum* L.) (WC), wheat-faba bean (*Vicia faba* L.) (WFb), wheat-sunflower (*Helianthus annuus* L.) (WS), and continuous wheat (WW)]. Sub-subplots were N-fertiliser rates (0, 50 and 150 kg N ha^{-1}) applied to wheat only (no other fertiliser applied to other crops). The area of each subplot was 50 m^2 (10 m × 5 m). More details about the management of tillage systems and soil sampling and analysis can be found in Melero et al. (2011).

We only considered the following depths: 0-5, 10-30, and 30-50 cm, for calculation of stratification ratio. Stratification ratio of SOM fractions and enzymatic activities was calculated based on the concentration of a soil property at a depth of 0-5 cm divided by the concentration of that property at a depth of 10-30 cm (SR1) and 30-50 cm depth (SR2) (Franzluebbers, 2002).

2.2. Statistical analysis

Data for each variable were subjected to analysis of variance using a randomized complete block design according to McIntosh (1983). Tillage was tested with tillage × block as error term; crop rotation and tillage × crop rotation were tested with tillage × crop rotation × block as error term; N fertiliser and its interactions with tillage and rotation were tested with tillage × rotation × N fertiliser × block as error term, and depth increment of stratification ratio and its interactions with tillage, rotation, and N fertiliser were tested with residual variation. Means were compared using Fisher's protected least significant difference (LSD) test at $P < 0.05$, using Statistix v. 8.1 (Analytical Software, 2005).

Relative importance of treatment factors on variation in response variables was assessed by adding the mean-square estimates for each of the four treatment factors (i.e. tillage, crop rotation, N fertiliser, and depth increment) and dividing by the sum across treatment factors to obtain a proportional estimate.

3. Results

Tillage system had a significant effect on stratification ratio of total N, WSC, AC, DHA and BGA, with the highest ratios under NT (Table 1). Stratification ratio [average of 0-5 / 10-30 cm (SR1) and 0-5 / 30-50 cm (SR2)] was 1.5 under NT and 1.3 under CT for total N, was 1.3 under NT and 0.9 under CT for WSC, was 1.03 under NT and 1.0 under CT for AC, was 1.5 under NT and 1.1 under CT for DHA, and was 3.2 under NT and 1.4 under CT for BGA. Stratification ratio of SOC was not different between tillage systems and averaged 1.4 for SOC across tillage systems.

Crop rotation significantly influenced the stratification ratio of most measured properties, except DHA (Table 1). Stratification ratio (average of SR1 and SR2) followed the order: WFb (1.5) = WW (1.5) > WF (1.4) = WC (1.3) = WS (1.2) for SOC, WFb (1.5) = WW (1.5) > WC (1.3) = WS (1.3) = WF (1.3) for total N, WW (1.4) = WFb (1.3) > WS (1.1) = WF (1.0) = WC (1.0) for WSC, WW (1.02) = WFb (1.02) > WF (1.01) = WC (1.00) = WS (1.00) for AC, and WFb (2.7) = WW (2.6) = WF (2.5) > WC (1.8) = WS (1.8) for BGA. A significant interaction occurred between tillage system and crop rotation for total N, WSC, AC and BGA (Table 1). Significant interactions were due to greater stratification ratio of total N, WSC and AC with WFb and WW under NT than under other crop rotations under either tillage system. Stratification ratio of BGA was greater under NT than under CT (3.8-2.4 vs 1.7-1.2) for all crop rotations, being the differences between both tillage systems higher in WW, WFB and WF than in WC and WS.

Nitrogen fertiliser rate affected the stratification ratio of BGA only (Table 1). Stratification ratio of BGA was 2.1 under 0 kg N ha^{-1}, was 2.2 under 50 kg N ha^{-1}, and was 2.7 under 150 kg N ha^{-1}. There was significant interaction between N fertiliser rate and other management features, and that was tillage × N fertiliser rate for stratification ratio of BGA and tillage × rotation × N fertiliser rate for stratification ratio of WSC (Table 1). Stratification ratio of BGA was 1.4 and 2.6 with 0 kg N ha^{-1} under CT and NT, respectively, was 1.3 and 3.0 with 50 kg N ha^{-1} under CT and NT, and was 1.4 and 3.9 with 150 kg N ha^{-1} under CT and NT. Greater stratification ratio of WSC occurred with WW compared with other crop rotations under NT at 50 and 150 kg N ha^{-1}.

Stratification ratio was significantly affected whether the denominator was 10-30 cm (SR1) or 30-50 cm (SR2) for SOC, total N, AC, WSC, DHA and BGA (Table 1, Figure 2). Greater stratification ratio occurred when calculated as 0-5 / 10-30 cm than as 0-5 / 30-50 cm for total N (1.5 vs 1.3, respectively), but the reverse effect occurred for SOC (1.3 vs 1.5, respectively), AC (1.01 vs 1.02, respectively), WSC (1.1 vs 1.2, respectively), DHA (1.2 vs 1.4, respectively), and BGA (1.7 vs 2.9, respectively). A significant interaction occurred between tillage system and stratification ratio with different denominator for SOC, total N and BGA (Table 1, Figure 2a). Stratification ratio of SOC was similar between tillage systems using SR2, but was greater under NT than under CT using SR1. Stratification ratio of total N was higher under NT than under CT in both SR1 and SR2. Stratification ratio of BGA was greater under NT than under CT using SR1 (2.3 vs 1.1, respectively), but was even more dramatically greater using SR2 (4.0 vs 1.7, respectively).

Crop rotation showed a significant interaction with stratification ratio with different denominator for SOC, WSC, AC, and BGA (Table 1, Figure 2b and c). Separation of WFb and WW from WC and WS with stratification ratio of SOC, WSC, AC, and BGA,

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as indicated earlier in the crop rotation main effect, was stronger using SR2 than using SR1. For stratification ratio of AC, there was no clear separation between these two groups of crop rotation using SR1. Significant interaction between tillage, rotation and stratification ratio with different denominator was observed for total N (Table 1). Greater stratification ratio of total N with WFb and WW compared with WF, WC, and WS occurred under NT using both stratification ratios, but no difference occurred between crop rotation groups under CT.

There was significant interaction between N fertiliser rate and stratification ratio with different denominator for AC, DHA and BGA (Table 1, Figure 2c). The magnitude of difference in stratification ratio of AC, DHA and BGA among N fertiliser rates was greater using SR2 than using SR1.

Significant interaction between tillage, N fertiliser and stratification ratio with different denominator was observed for total N (Table 1, Figure 2d). Greater stratification ratio of total N under NT than under CT at 0, 50 and 150 kg N ha^{-1} occurred using SR1, but these effects were subdued using SR2.

There was no interaction between any combinations of rotation $\times$ N fertiliser, tillage $\times$ crop rotation $\times$ N fertiliser rate and stratification ratio with different denominator.

In general, stratification ratio of most properties was < 2 for all treatments, except the stratification ratio of BGA, which had ratios > 2 under NT for all crop rotations.

Relative importance among treatment factors in explaining variations in stratification ratio was different depending upon response variable. Relative proportion of variation associated with treatment factors for all studied parameters is shown in Fig. 3. Summarized across the six response variables, tillage treatment explained 54 $\pm$ 15% of the variation, crop rotation explained 13 $\pm$ 7% of the variation, N fertiliser rate explained

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$8 \pm 3\%$ of the variation, and depth increment of stratification ratio explained $25 \pm 14\%$ of the variation.

4. Discussion

In our study, stratification ratio of total N was higher under NT than under CT especially when using 0-5 / 10-30 cm (SR1), whereas stratification ratio of SOC and BGA tended to be greater under NT in both SR1 and 0-5 / 30-50 cm (SR2), indicating the effectiveness of NT in improving C and N accumulation and biological status of the surface layer. This result supported our hypothesis that higher accumulation of crop residues under no tillage management would increase pools of organic matter and BGA at upper layers. Stratification ratio of SOC was similar between tillage systems using SR2, but was greater under NT than under CT using SR1. Therefore, our hypothesis for obtaining greater stratification ratio using SR2 than using SR1 was not supported. The lack of strong difference in stratification ratio could reflect some C and N accumulation in the sub-surface layer under CT derived from inversion of soil with tillage and under NT from the self-tilling characteristic of this high-clay content Vertisol. In a previous study carried out in the same region and on the same soil type, Melero et al. (2008) found no difference between CT and NT treatments for stratification ratio of SOC and enzymatic activities (dehydrogenase, β-glucosidase, arylsulphatase and phosphatase activities), which were always < 2, when considering the 10-20 cm depth as the denominator. In a clayey soil in Alberta, Canada, Franzluebbers (2002) found no statistical difference between CT and NT in SOC, particulate organic C, soil microbial biomass C, and potential C and N mineralization stratification ratios. However, coarse-textured soils were generally lower in degree of aggregation and SOM, and therefore responded more significantly to NT compared with CT from binding agents. In addition, Jarecki and Lal (2005) found higher

SOC and N stratification ratio (0-5 / 15-30 cm) in NT (3.7 for SOC and 3.2 for N) than in chisel tillage (1.5 and 1.4, respectively) and mouldboard ploughing (1.2 and 1.2, respectively) in a silt loam soil, but stratification ratio of SOC was low and not significantly different among treatments in a clayey soil (1.4 under NT, 1.3 under chisel tillage, and 1.1 under mouldboard ploughing).

As pointed out by Franzluebbers (2002), there is an enormous task to accurately define the minimum and maximum levels of soil properties under the multitude of climatic regions and different soil properties with an eye towards identifying the effect of management on soil ecosystem functioning. Nevertheless the importance of soil texture (a variable related to soil richness) should be considered when using stratification ratio as an useful index of the impact of soil management on soil quality. Noellemeyer et al. (2006) also found a positive relation between texture and SOC in a wide range of soils, possibly due to the stabilization of organic compounds by clay particles. Few studies measuring stratification ratio have been conducted in soils with as high of clay content as studied here (700 g kg^{-1} clay). In our case, results could be more related to the characteristic of Vertisols, which are comparatively rich in SOM and contain expandable mineral clays that swell upon re-wetting and shrink upon drying, forming wide cracks, which could allow for a better mixing of organic materials and soil particles in soil profile. Such self-tilling soils would reduce concentration gradients typically found in NT systems (Dalal et al., 1991). Therefore, in these types of soils, deeper layers (e.g., 30-50 cm, below the tilled horizon) than those frequently used as a denominator might be necessary to show benefits derived from conservation tillage and other good agricultural practices.

In addition, in general, we observed that stratification ratio of most studied variables, including SOC were < 2 in both CT and NT systems for both stratification ratios (0-5 /

10-30 cm and 0-5 / 30-50 cm), with the exception of BGA. Stratification ratio of BGA was clearly > 2 in NT, especially at 0-5 / 30-50 cm in all crop rotations and in all N doses. Thus, under our conditions, BGA could be considered a sensitive soil quality indicator, reflecting an improved environment introduced by conservation tillage. However, stratification ratio > 2 may not have to be considered a threshold value to indicate the sustainability of an agricultural system. In our study, stratification ratio < 2 found for many C and N fractions could be more related to the self-tilling characteristic of Vertisols. Our results corroborated our hypothesis showing enhanced stratification ratio of soil organic fractions and BGA with more intensive crop rotations than under wheat-fallow, which is consistent with a study showing enhancement of biologically active soil C and N fractions (soil microbial biomass and potential activity) with increasing cropping intensity and greater C inputs (Franzluebbers, 2002). The importance of crop management in affecting stratification ratios under semi-arid, rainfed conditions has been emphasised previously. Álvaro-Fuentes et al. (2008) reported greater stratification ratio in wheat-barley-wheat-rapeseed rotation than in continouos barley or barley–fallow rotation for all tillage treatments (5.1 vs 1.7 under NT, respectively, and 2.0 and 1.0 under CT, respectively). Hernanz et al. (2009) reported that a change of cereal-fallow rotation to wheat-legume (vetch and pea) rotation increased stratification ratio (0-10 / 30-40 cm) of SOC, as well as changing from CT (1.4) to chisel tillage (2.0) or to NT (2.6), indicating an improvement in soil quality. In our study, WFb and WW had significantly greater stratification ratio of SOC and total N than other rotations, especially using SR1 under NT. However, only BGA had SR2 > 2 in WFb, WW, and WF, compared to other crop rotations, especially in NT. These results could be associated with higher above-ground residue yields of faba bean and wheat than in other rotations under NT, but not under CT (Melero et al., 2011). Lower stratification ratios were observed under WC and WS

rotations, due to low production of above-ground residues of chickpea and low degradation rate of sunflower residue (Melero et al., 2011). N fertilisation rate may have influenced stratification ratio of total N due to higher wheat grain yield and above ground residues of wheat, especially under NT using SR1 (Melero et al., 2011). In general, three doses of N fertiliser (0, 50 and 150 kg N ha^{-1}) had similar influence in stratification ratio of AC, DHA and BGA, which were higher using SR2 than using SR1. These results are probably due to the similar inputs of above ground residues of wheat in plots with different N fertiliser rates (Melero et al., 2011).

Conclusions

Tillage system and crop rotation were more important than N fertilizer rate in affecting stratification ratio of C and N fractions and enzymatic activities. Intensive crop cultivation, especially WW and WFb, improve soil fertility by an increase of accumulation of soil organic fractions and BGA in upper layers in comparison with deeper layers in both tillage systems. Stratification ratio of β-glucosidase activity (>2) was the best indicator for assessing the improvement of conservation tillage on soil quality under these semi-arid Mediterranean conditions. However, stratification ratio > 2 might not have to be considered a threshold value to indicate the sustainability of an agricultural system, since textural characteristics, in our case of dryland Vertisols, have a great influence in lack of strong difference between stratification ratios and between tillage systems.

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Table 1. Analysis of variance (mean squares) of the stratification ratios for different parameters as affected by tillage system, crop rotation, N rate and the depth considered for the stratification ratio.

Source	df	SOC	Total N	WSC	AC	DHA	BGA
Tillage (T)	1	1.44	1.29*	6.33*	$16\ 10^{-3}$**	7.92**	141***
Error a	4	0.29	0.08	0.46	$4\ 10^{-4}$	0.23	1.1
Rotation (R)	4	0.73***	0.26***	1.24***	$15\ 10^{-4}$***	0.02	6.7***
T × R	4	0.1	0.24***	0.76**	$9\ 10^{-4}$**	0.09	2.8*
Error b	16	0.07	0.03	0.15	$2\ 10^{-4}$	0.14	0.7
Nitrogen (N)	2	0.19	0.09	$3\ 10^{-3}$	$7\ 10^{-5}$	0.39	5.05*
T × N	2	0.14	$5\ 10^{-3}$	0.16	$6\ 10^{-5}$	0.03	6.6*
R × N	8	0.04	0.05	0.22*	$2\ 10^{-4}$	0.22	1.7
T × R × N	8	0.13	0.05	0.33**	$1\ 10^{-4}$	0.14	2.4
Error c	40	0.08	0.03	0.1	$2\ 10^{-4}$	0.17	1.5
Stratification ratio (SR)	1	3.12***	0.87***	0.51***	$3\ 10^{-3}$***	1.87***	62.3***
T × SR	1	0.21*	0.1**	0.03	$2\ 10^{-5}$	$1\ 10^{-3}$	15.8***
R × SR	4	0.11*	0.02	0.31***	$3\ 10^{-4}$***	0.12	5.3**
N × SR	2	0.11	$1\ 10^{-4}$	0.08	$2\ 10^{-4}$**	0.47**	3.4*
T × R × SR	4	0.06	0.03*	0.02	$4\ 10^{-5}$	0.03	2.4
T × N × SR	2	0.09	0.06*	0.02	$5\ 10^{-5}$	0.02	2.4
R × N × SR	8	0.02	$4\ 10^{-3}$	0.04	$2\ 10^{-5}$	0.07	1.1
T × R × N × SR	8	0.04	$9\ 10^{-3}$	0.05	$4\ 10^{-5}$	0.05	1.4
Error d	60	0.04	0.01	0.03	$3\ 10^{-5}$	0.07	1.1

SOC: soil organic carbon; WSC: waters soluble carbon; AC: active carbon; DHA: dehydrogenase activity; BGA: β-glucosidase activity.
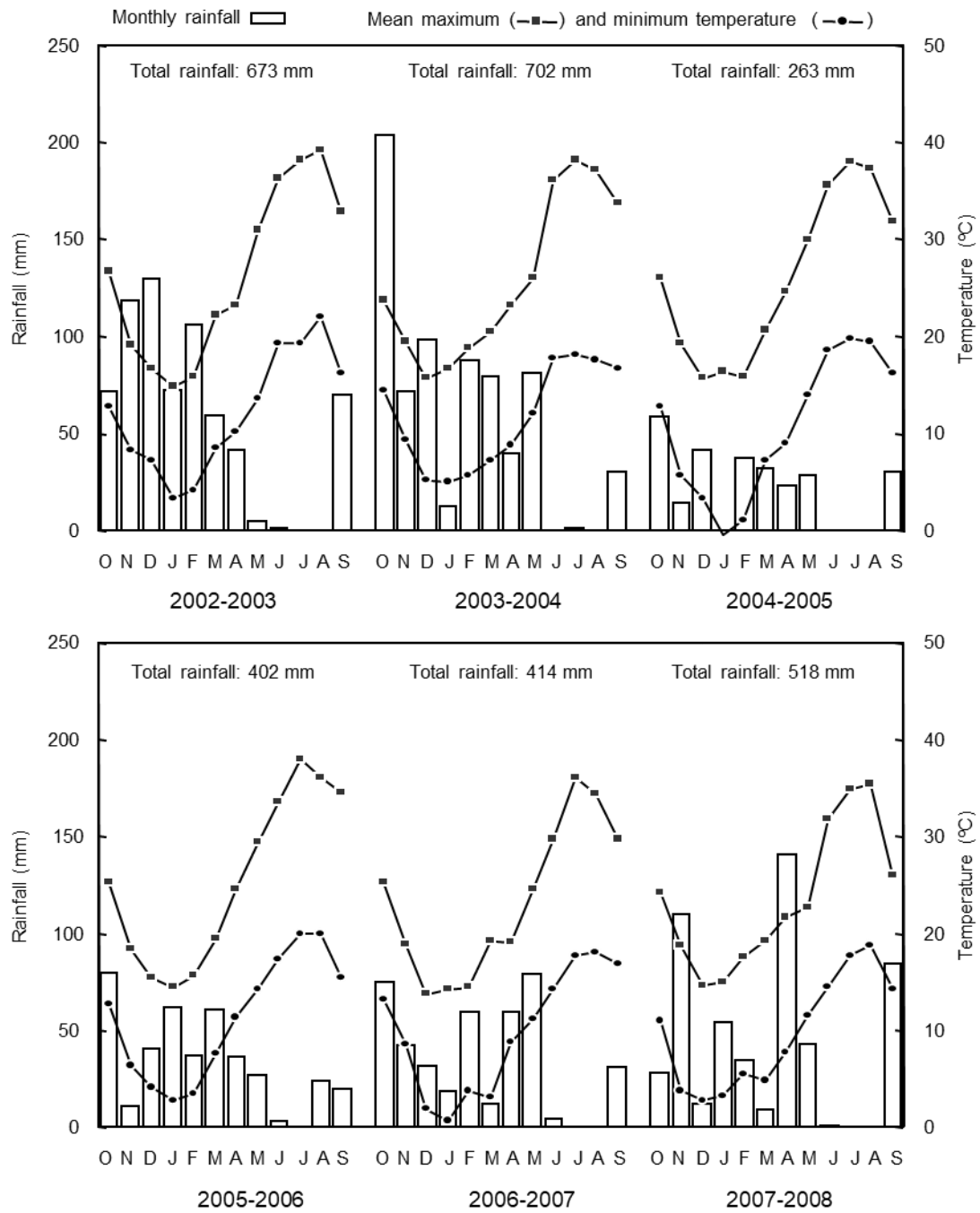(*) ($p < 0.05$); (**) ($p < 0.01$); (***) ($p < 0.001$)

Figure 1. Mean maximum and minimum temperature and rainfall for experimentation time.
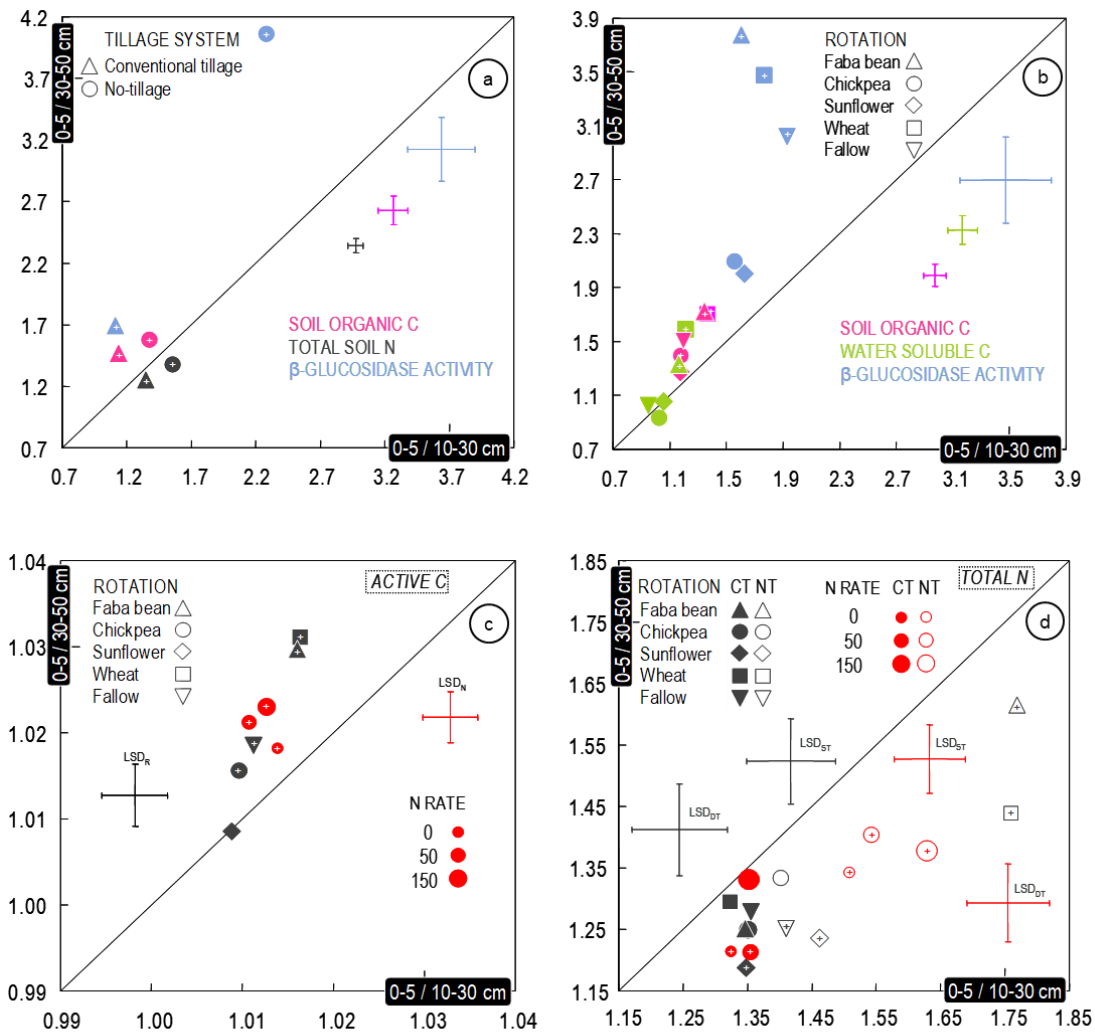
Figure 2. Interactions between stratification ratios (0-5/10-30 and 0-5/30-50 cm) with: (a) tillage system (NT, no-tillage; CT, conventional tillage) on SOC, total N and BGA, (b) crop rotation on SOC, WSC and BGA, (c) crop rotation and N rate on AC, (d) tillage system × crop rotation and tillage system × N rate on total N. Symbols (triangle, square, circle and rhombus) marked with cross represent significant differences between stratification ratios. Symbols with different colour represent different soil parameters in figure 2a and b (pink, SOC; grey, total N; green, WSC; blue, BGA) whereas they represent different management in figure 2c and d (grey, rotation; red, N rate). The same criterion of colours has been used for cross LSD. In figure 2d solid and empty symbols represent CT and NT, respectively. Cross LSD shows its magnitude (at p <0.05) in horizontal and vertical to compare means for: (a) different level of tillage, (b) different level of rotation, (c) different level of rotation (LSD$_R$) and different level of N rate (LSD$_N$), (d) the same level of tillage (LSD$_{ST}$) and different level of tillage (LSD$_{DT}$).
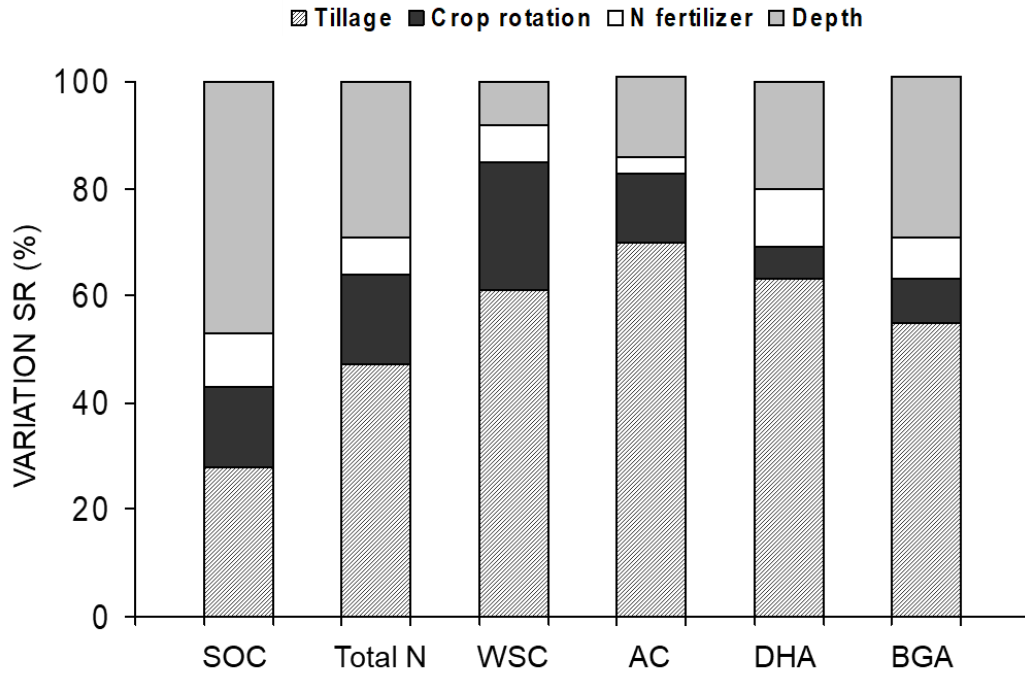
Figure 3. Relative proportion of variation in stratification ratios (SR) associated with treatment factors for all studied parameters. (SOC: soil organic carbon; WSC: water soluble carbon; AC: active carbon; DHA: dehydrogenase activity; BGA: β-glucosidase activity)