The impact of crop diversification, tillage and fertilization type on soil total microbial, fungal and bacterial abundance: A worldwide meta-analysis of agricultural sites

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ABSTRACT

Microorganisms play a key role in nutrient cycling in agriculture and can contribute to improve soil quality and enhance crop production. Thus, there is a need to identify the most suitable management practices which foster increases in soil microbial biomass and diversity. A meta-analysis was performed to assess changes in microbial abundance in agricultural soils affected by: (i) management practices (tillage, fertilization and crop diversification); and (ii) environmental factors, including climate characteristics and soil properties. The scope of the meta-analysis was to evaluate whether microbial abundances are affected or not by organic fertilization or no fertilization, crop diversification (intercropping and crop rotations) and conservation tillage (reduced tillage/no-tillage) as an alternative to intensive conventional monocultures in agriculture. Only papers showing data on phospholipid fatty acids (PLFAs), providing indicators about soil microbial (total PLFA), fungal and bacterial biomass reached a critical mass to perform the meta-analysis. Therefore, soil microbial diversity could not be analyzed considering different management practices. Results showed that intercropping and crop rotations only significantly increased the abundance of fungi, with the corresponding increase in the fungal-to-bacterial ratio. Organic fertilization contributed to significant increases in bacterial and fungal abundance and total PLFA compared to mineral fertilization. Contrarily, the lack of fertilization negatively affected total PLFA, with no significant effect on bacterial and fungal abundances. Reduced tillage significantly increased total PLFA, fungal and bacterial abundances compared to conventional tillage, while no tillage had only a positive effect on fungi. Thus, as a general pattern, the adoption of sustainable management practices, mostly organic fertilization and reduced tillage, has overall positive effects on soil total microbial, fungal and bacterial abundance. These variables were not related to soil physicochemical properties and climatic factors, suggesting a positive global effect of sustainable management practices on soil microbial abundances. Thus, this study shows new insights by a meta-analysis of global studies about the effect of sustainable management practices on soil microbial abundances, needed for land-managers, policy-makers and farmers to select sustainable cropping systems that enhance microbial abundance.

1. Introduction

The challenge to produce food and fiber for the global population, expected to reach between 9 and 11 billion people by 2050, may intensify agriculture (FAO, 2017). To ensure food security and protect the environment, sustainable agricultural practices should be adopted urgently. Soil health and functionality must be taken into account as they also constitute a challenge to the near future (European Commission, 2021). Moreover, soil microbes are of high importance as they are the cornerstone to preserving soil functions and soil ecosystem goods and services (FAO et al., 2020). The implementation of green and suitable agricultural management practices may be an alternative to reduce

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pressure in current agriculture and maintain or even enhance soil microbial abundance. Thus, crop diversification has shown benefits for soil macro- and microorganisms while maintain crop yields (Rodríguez et al., 2020; Tamburini et al., 2020; Zhang et al., 2020; Redlich et al., 2021), and it is well known that the application of organic fertilizers instead of inorganic fertilization reduces losses of nutrients, maintaining their bioavailability; decreases water pollution, increases SOC stocks, while stabilizing resources for soil biology (Diacono and Montemurro, 2010; Scotti et al., 2015). Conservation tillage, such as no-tillage or reduced tillage, are considered agricultural conservation practices which decrease soil disturbance, protect the soil against erosion and increase soil organic matter (Doran, 2002). Recent studies also show that conservation tillage protects life cycles of arthropods, increasing their diversity (Mhlanga et al., 2020), and plays a major role in shaping microbial communities (Kraut-Cohen et al., 2020).

Meta-analysis is a quantitative synthesis methodology enabling establishment of the effect-size of a population (Sánchez-Meca and Marín-Martínez, 2010), i.e., estimating global results to answer fundamental questions. For instance, a meta-analysis study indicated that intensifying agriculture may significantly alter soil biodiversity, having negative effects on several microbial groups but also positively affecting fungal- and microbial functional diversity (de Graaff et al., 2019). Related to the implementation of sustainable agricultural management, Tamburini et al. (2020) revealed how diversifying agriculture increases soil fertility, nutrient cycling, pest control, biodiversity and water regulation, yet does not compromise crop yields. Rotation and intercropping are diversification strategies associated with increased yields while agroforestry brings about improvements in biodiversity as well as soil quality (Beillouin et al., 2019). Crop diversification also showed higher microbial richness and diversity (Venter et al., 2016). Conservation agriculture has shown that plant cover in the alleys of vineyards adds to conserving biodiversity and providing numerous ecosystem services (Winter et al., 2018). However, a more recent study has shown that no-tillage increased soil bacterial diversity while had no effect on soil fungal diversity (Li et al., 2020a). Moreover, the application of inorganic N fertilization affects bacterial diversity and community composition (Dai et al., 2018).

The study of soil microbial biomass and the characterization of soil microbial communities are usually determined by PLFA technique, using the lipids of the microorganism's membrane cells as biomarkers, since they are only found in viable microbes. Changes in PLFA patterns are indicative of rapid changes in microbial community structure while the total amount of PLFA has been used to determine total microbial biomass (Frostegård et al., 1991; Baath et al., 1992; Zelles, 1999). Specific PLFA may be used to determine microbial biomass of different groups, such as bacterial PLFA and fungal PLFA (Frostegård and Bååth, 1996). The use of fungal/bacterial PLFA ratio is a parameter indicative of a sustainable and self-regulated system (Bardgett and McAlister, 1999; van Groenigen et al., 2010). Thus, knowing the abundance of the bacterial and fungal groups gives information about the characteristics and functioning mechanisms of soil microbiology. More recently, RNA and DNA techniques were implemented in order to measure the diversity of microbial communities giving information about their composition, richness and structure (Dunbar et al., 1999; Rondon et al., 2000; Zhang and Xu, 2008).

Up to date, meta-analysis studies have analyzed the effects of just one sustainable agricultural practice compared to the conventional one on microbial abundance. However, no studies have been published analyzing not only the effect of different sustainable management practices together on microbial abundance but also the effects of environmental characteristics. In our study, a methodical literature search was performed followed by a worldwide meta-analysis that aimed to: i) evaluate the effect of crop diversification, fertilization type and tillage on soil total microbial, fungal and bacterial abundance, and ii) elucidate if soil physicochemical properties and climatic factors condition the response of soil microbial abundances to the sustainable management

practices. We therefore contrasted the use of crop rotations (growing different crop species on the same land in successive growing seasons) and intercropping (growing different arable crop species in proximity, in the same field, via mixed, row and strip) related to monocultures (Lloret et al., 2020); the use of organic fertilizers (manure/compost/sludge, green manure) or no fertilization (no application any type of fertilizer on soil) compared to mineral or inorganic fertilizers; and the implementation of minimum tillage or no-tillage related (non-plough based cultivation practices, seed is usually sown with discs working to 10–15 cm or rotary-harrowing of the soil surface) to conventional tillage (ploughing to a depth of at least 20 cm followed by secondary practices such as harrowing or discing/tining) (Powlson et al., 2012). To reach those goals, a meta-analysis with the results obtained in 77 papers was conducted. We hypothesized that soil total microbial, fungal and bacterial abundances would increase with sustainable management practices such as crop diversification, organic fertilization or reduced tillage, compared to conventional management, independently of crop type, soil type and climatic factors.

2. Materials and methods

2.1. Data collection

To assess the effect of some sustainable management practices compared to conventional management on soil microbiological properties, a literature search was conducted in SCOPUS, SCIENCEDIRECT, WEB OF SCIENCE and GOOGLE SCHOLAR, seeking peer-reviewed articles published online until July 2020. The resulting database consisted of 393 experimental treatments from 77 papers from different nations: Spain, Italy, Germany, Belgium, United Kingdom, Argentina, Switzerland, Sweden, Finland, China, Brazil, Turkey, Indonesia, South Africa, Kenya, Japan, Korea, Canada and the United States of America (Fig. 1). Studies comprised the following Köppen climatic zones (A to D): tropical (18 studies), arid (6 studies), temperate (30 studies) and continental (24 studies). To retrieve the articles listed, the following keywords were employed: "conventional AND organic AND microbial AND indicators AND agricultural soil AND PLFA", "AND" any combination of the items: "Cover crop", "intercropping", "rotation", "biodiversity", "fungal", "bacteria", "qPCR", "tillage", "fertilization", "Simpson index", "Shannon index", "Richness index", "ITS fungal", "total DNA", "Arbuscular mycorrhizal", "16 S" and "16 S rRNA". Any study conducted in a laboratory or a greenhouse was not included. Only studies carried out in field conditions were chosen, regardless of crop type, soil type and climate. The initial idea was to only focus on arable crops, but not enough papers were found to perform a meta-analysis with a significant number of interactions. Peer-reviewed scientific papers were eligible and included in our meta-analysis with the criteria explained in Fig. 2.

A database was built up containing the relevant data pertaining to (i) management (fertilizers type, tillage, crop diversification); (ii) environmental factors like soil type and climate variables (mean annual temperature and precipitation), (iii) study length (in years), (iv) soil properties (clay, pH, organic carbon and total nitrogen) and (v) response variables (16 S Bacterial Simpson index * , 16 S Bacterial Shannon index * , 16 S Chao richness index * , ITS fungal Simpson Index * , ITS Chao richness * , ITS Fungal Shannon index * , 16 S rRNA copies * , ITS gene copies * , total DNA * , Arbuscular mycorrhiza fungi * , Total PLFA, Fungal PLFA, Bacterial PLFA and fungal-to-bacterial PLFA ratio). Few articles showed values of the * marked variables, so we did not achieve a critical mass to perform a meta-analysis (n > 8) (Lee et al., 2019). Hence, the selected properties to be used in this meta-analysis were bacterial PLFA, fungal PLFA and total PLFA. The database can be consulted in Excel spreadsheet format at Zenodo repository (Morugán-Coronado et al., 2021) (https://zenodo.org/record/4884673#. YLURNqHtbqV), with indication of soil depth utilized, specific soil characteristics and additional information about agricultural management. The crops used for the study were mostly maize (*Zea mays*) with
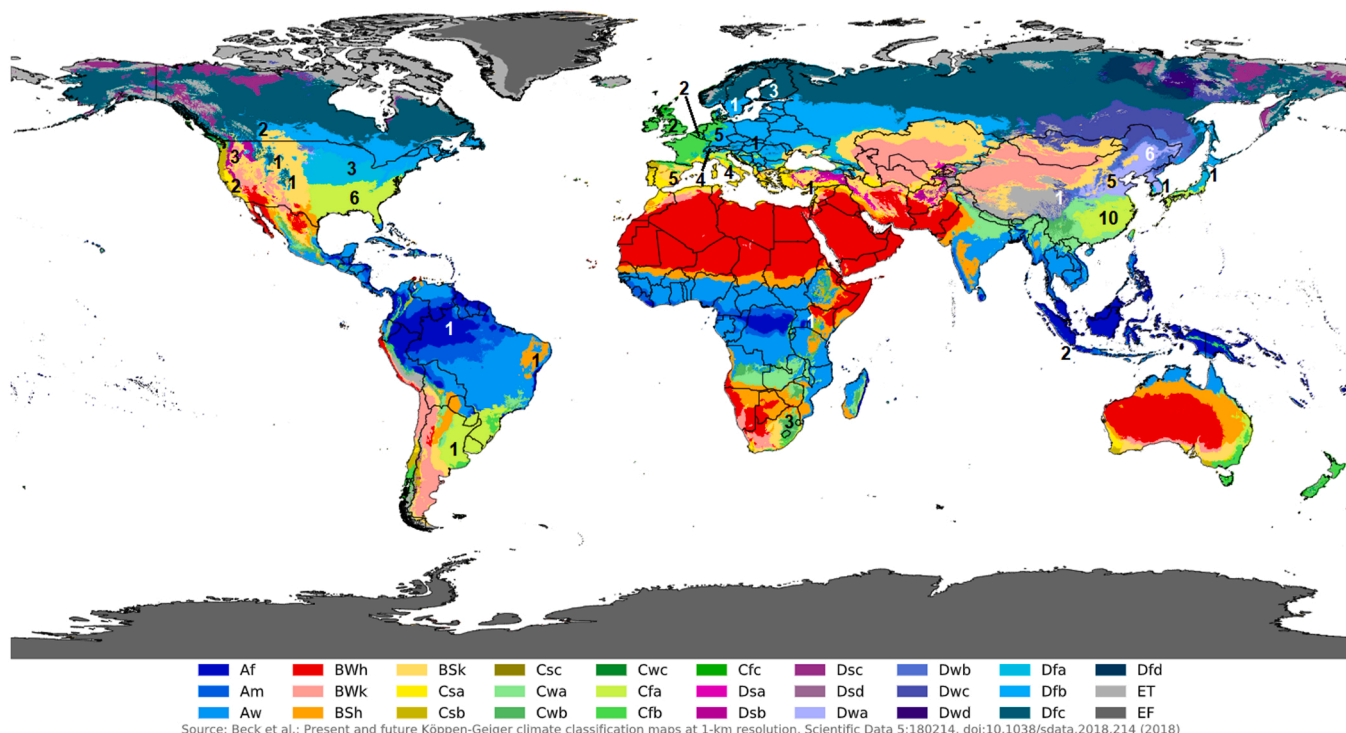
Fig. 1. Map of the studies included in this meta-analysis classified by Köppen-Geiger Climate zones (1980–2016). Numbers indicate the total number of articles per country.
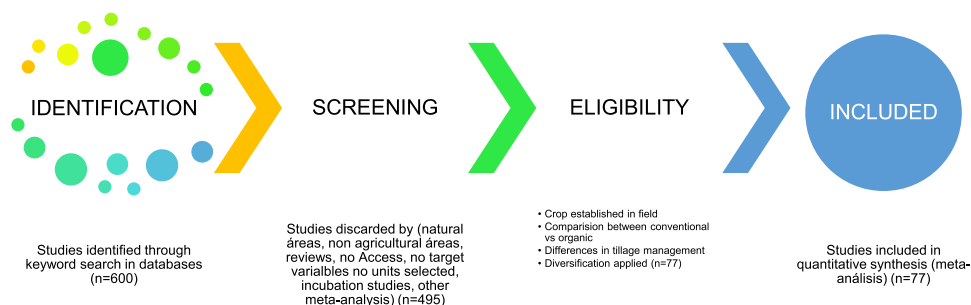Map modified from Beck et al. (2018).

Fig. 2. The chart shows the overview of identified, excluded, and included studies in this meta-analysis.
PRISMA flow diagram modified from Lori et al. (2017).

37% of sample size, wheat (*Triticum aestivum*) with 25% of sample size, tomato (*Solanum lycopersicum*), rice (*Oryza sativa*) and soybean (*Glycine max*) with 8% of sample size each crop, barley (*Hordeum vulgare*) with 7% of sample size, cotton (*Gossypium hirsutum*) with 3% of sample size, pepper (*Capsicum annuum*) with 2% of sample size, and potato (*Solanum tuberosum*) and wild cabbage (*Brassica oleracea*), representing 1% of the total dataset. The main fruit trees were grapevine (*Vitis vinifera*), almond trees (*Prunus dulcis*) and olive trees (*Olea europaea*) with 1% of size.

To assess the effect of crop diversification, tillage intensity and fertilizer type on bacterial PLFA, fungal PLFA, fungal-to-bacterial ratio (F: B) and total PLFA, we considered the following categories one-by-one (percentages in parentheses denote the frequency of each sub-group within a category):

A. Diversification type, which compares intercropping (IC) (12%) and crop rotations (ROT) (88%) versus monocultures. Crop rotations indicate the technique of planting a series of different crop types in a particular zone over a number of cropping seasons. Intercropping indicates the cultivation of two or more crops simultaneously in time and space. Mono-cropping involves the growth of only one cash crop and the absence of rotations or intercropping and no use of cover crops.

B. Fertilization type, comparing no fertilization (NF) (45%) and organic fertilizer (Org) (55%) as opposed to conventional mineral fertilizer. Organic fertilizers are constituted of animal-based or plant matter which may be a by-product or end product of a process that occurs naturally; this may include compost, sludge, humic acids, manure or green manure. Inorganic fertilizers are chemically made with the presence of synthetic chemicals or minerals. Organic practices were managed for 19.2 years on average and conventional sites were managed for 17.2 years on average.

C. Tillage intensity, comparing reduced tillage (RT) (25%) and no-tillage (NT) (75%) against conventional tillage.

2.2. Statistical analysis

The effect of tillage intensity, crop diversification and fertilizers on soil microbiological properties based on PLFA extraction and quantifi-

cation was studied by means of a meta-analysis. The response ratio was determined as the effect size unit for each indicator (Aguilera et al., 2013). Research in agriculture has extensively utilized that ratio for meta-analyses (e.g., Aguilera et al., 2013; Lee et al., 2019; Li et al., 2018). The response ratio (RR) was computed as percentage variations of the indicator value of the treatment group (sustainable management: rotation/intercropping, organic fertilization, no-tillage/minimum tillage) ($\underline{X}_{SM}$) versus the pair-wise control group (conventional management: mono-cropping, inorganic fertilization, conventional tillage) (($\underline{X}_C$)) (Eq. 1):

$$RR = \frac{\underline{X}_{SM}}{\underline{X}_C} \tag{1}$$

The analysis utilized the natural logarithm of the response ratio (log (RR)) (Eq. 2). That transformation linearized the metric into small samples (Hedges et al., 1999).

$$\text{Log(RR)} = ln\frac{\underline{X}_{SM}}{\underline{X}_C} = \ln(\underline{X}_{SM}) - \ln(\underline{X}_C) \tag{2}$$

Positive values represent higher values of sustainable management, whilst negative values show higher values of conventional management. In meta-analyses, weightings are typically given through the inverse of their variance, yet such information was lacking in several of the works found. Our decision was to weight observations by sample size in order to guarantee statistical significance by ensuring a sufficient sample size: larger sample sizes were given a higher weighting during aggregation (Adams et al., 1997). The weighted log (RR) (Wlog(RR)) of management option i was determined with Eq. 3:

$$\text{Wlog(RR)i} = \frac{1}{N_i}\Sigma(log(RR)_{ij} \quad x \quad W_{ij}) \tag{3}$$

where N_i is the number of studies for the management i, log(RR)$_{ij}$ is the log(RR) of management i in study j, and W_{ij} is the weight, Eq. 4:

$$\text{Wij} = \frac{N_i^{SM} \qquad N_i^C}{N_i^{SM} \quad + \quad N_i^C} \tag{4}$$

where N_i^{SM} and N_i^C represent the sample size of sustainable and conventional management of the management i in study j, respectively (Adams et al., 1997). As for Log (RR), Wlog(RR) with positive values indicating higher values in sustainable management, whilst negative values correspond to higher values in conventional management. In the uncertainty analysis, we detailed the means and 95% confidence intervals (CIs) of the Wlog(RR) (Adams et al., 1997; Davison and Hunkley, 1997; Lee et al., 2019). The CIs were built by non-parametric bootstrapping (nboot = 10 000). This bootstrapping was performed for treatments with n ≥ 8, since it becomes unreliable for overly small samples (Lee et al., 2019). An exception was made to compare the effect of intercropping on total PLFA, since we achieved 7 treatments and decided not to leave it out. The effect of treatment was deemed significant if there was overlapping of the 95% bootstrap CI with zero (Lee et al., 2019). Correlations and regressions between Wlog(RR) of total PLFA, fungal PLFA, bacterial PLFA and F:B ratio, and the variables soil clay, organic carbon, pH, total nitrogen, C:N ratio, mean annual precipitation, mean annual temperature and study length were performed to determine how the environmental factors and study length affect the response rate of the analyzed variables. IBM SPSS Statistics 24 Software was used for CI bootstrapping and statistical analyses. SIGMAPLOT was used to plot the weighted RR values.

3. Results

3.1. General results

From the 600 articles initially identified in the databases, 0 of them reported data on 16 S Bacterial Simpson index, 7 on 16 S Bacterial

Shannon index, 4 articles on 16 S Chao richness index, 2 on ITS fungal Simpson Index, 2 on ITS Chao richness, 2 on ITS Fungal Shannon index, 1 on 16 S rRNA copies, 0 on ITS gene copies, 2 on total DNA and 15 articles on Arbuscular mycorrhiza fungi. These articles provided < 8 experimental treatments, so meta-analysis had to be discarded. Only total PLFA, fungal PLFA, bacterial PLFA and F:B ratio provided enough experimental treatment to perform an accurate meta-analysis (n ≥ 8). Studies found dealing with total PLFA, fungal PLFA, bacterial PLFA and F:B ratio spanned from 1999 to 2020. However, articles dealing with metagenomic indicators spanned from 2011 to 2020, with 90% of them being concentrated in the period 2018–2020.

3.2. Influence of crop diversification

Crop diversification had no significant effect on bacterial PLFA nor total PLFA (Fig. 3A and Fig. 4B). Intercropping and rotation was nonetheless significant for fungal PLFA (Fig. 3B) with Wlog(RR) of 0.15 and 0.16, respectively. The use of intercropping was significant for F:B ratio (Fig. 4A), with Wlog(RR) of 0.45. Although crop diversification had no significant effect on bacterial PLFA and total PLFA compared to monocultures, 60–71% of values observed were positive, showing an overall positive effect. The values of Wlog(RR) were 0.23 and 0.28 for IC and ROT, respectively, in bacterial PLFA (Fig. 3A), and 0.24 and 0.19 for IC and ROT, respectively, in total PLFA (Fig. 4B). For F:B ratio, 705 of values observed with rotations was positive, despite the lack of a significant effect.

3.3. Influence of fertilization type

The practice of avoiding the use of fertilizers (NF) had no significant effect on bacterial PLFA, fungal PLFA and F:B ratio (Fig. 3A, B and Fig. 4A), although WlogRR for bacterial PLFA was negative (−0.07), some 68% of the studies showed negative effects. Moreover, the 64% of studies about fungal PLFAs showed a negative effect of NF compared to mineral fertilizers, with WlogRR = −0.15. There was a negative significant effect of NF on total PLFA, with WlogRR = −0.15%, and 69% of the studies showing negative effects (Fig. 4B). ORG was linked with a significant increase in bacterial, fungal and total PLFAs related to mineral fertilization (Fig. 3A, B and Fig. 4B). Such an increase was greater for total PLFA (WlogRR = 0.82) than for bacteria (0.54) and fungi (0.46), with 75–94% of the studies reporting positive effects. The F:B ratio was not significantly affected by ORG (Fig. 4A).

3.4. Influence of conservation tillage

RT management was associated with significantly increased bacterial, fungal and total PLFA as opposed to conventional tillage (Fig. 3A, B and Fig. 4B). The response ratios for total, fungal, bacterial were 0.69, 0.66 and 0.65, respectively, with > 80% of studies reporting positive effects. RT did not significantly affect the F:B ratio, even though 67% of values showed positive effects. NT presented a significant positive effect on fungal PLFA in comparison with conventional tillage (Fig. 3B), with Wlog(RR) = 0.25. However, the effect of reduced tillage was higher than the adoption of no tillage for fungi. NT showed no significant influence on bacterial PLFA, F:B ratio and total PLFAs (Fig. 3A and Fig. 4A, B), although the response ratio was slightly negative in bacterial and total PLFAs (Wlog(RR) = −0.05, − 0.04, respectively) and positive in F:B ratio (Wlog(RR)= 0.23).

3.5. Influence of climate, soil clay and study length

The response ratios determined for and total PLFA, fungal PLFA, bacterial PLFA and F:B ratio were not significantly correlated with the experimental length of the studies considered, soil clay, organic carbon, pH, total nitrogen, C:N ratio, temperature or precipitation (P > 0.05). Thus, no significant regression was achieved (Figs. S1–3 in
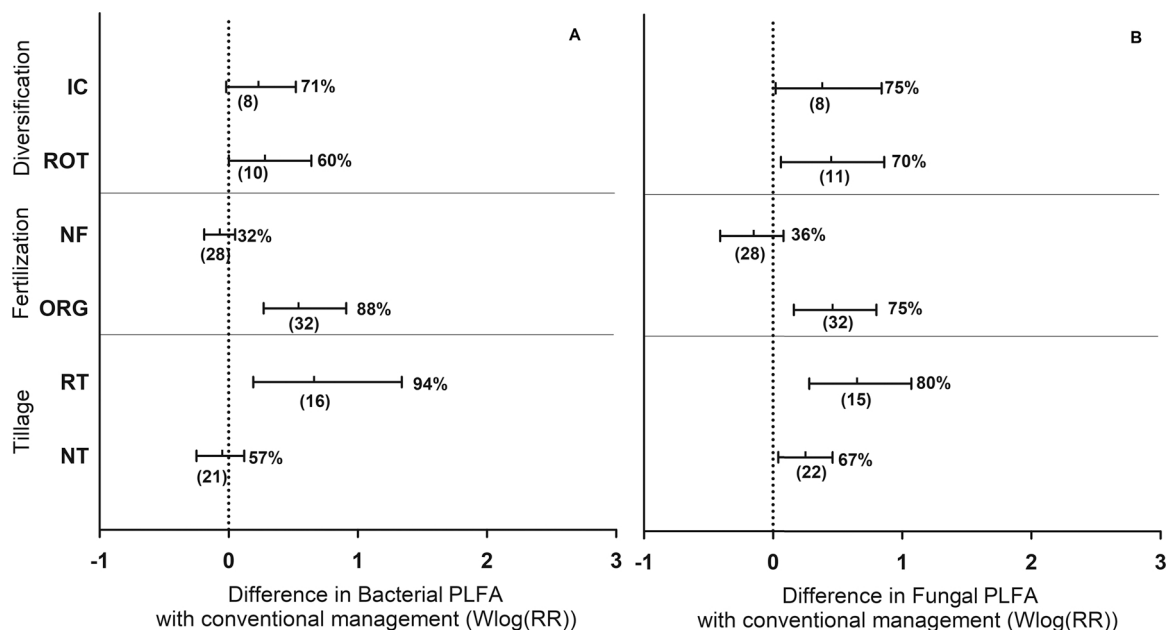
Fig. 3. Effect of diversification (IC: intercropping, ROT: rotations), fertilization (NF: no fertilization, ORG: organic fertilization) and conservation tillage (RT: reduced tillage, NT: no tillage) on bacterial PLFA (A) and fungal PLFA (B). Values are shown as weighted log response ratio: Wlog(RR). The "I" denotes the mean and the horizontal bar represents the 95% confidence interval. The number below each bar indicates the size of the sample. Y-axis levels with means > 0 show that treatment increased the soil microbial property and decreased if means are < 0. The values in percentages indicate the fraction of studies showing positive effects of that factor on the studied property.
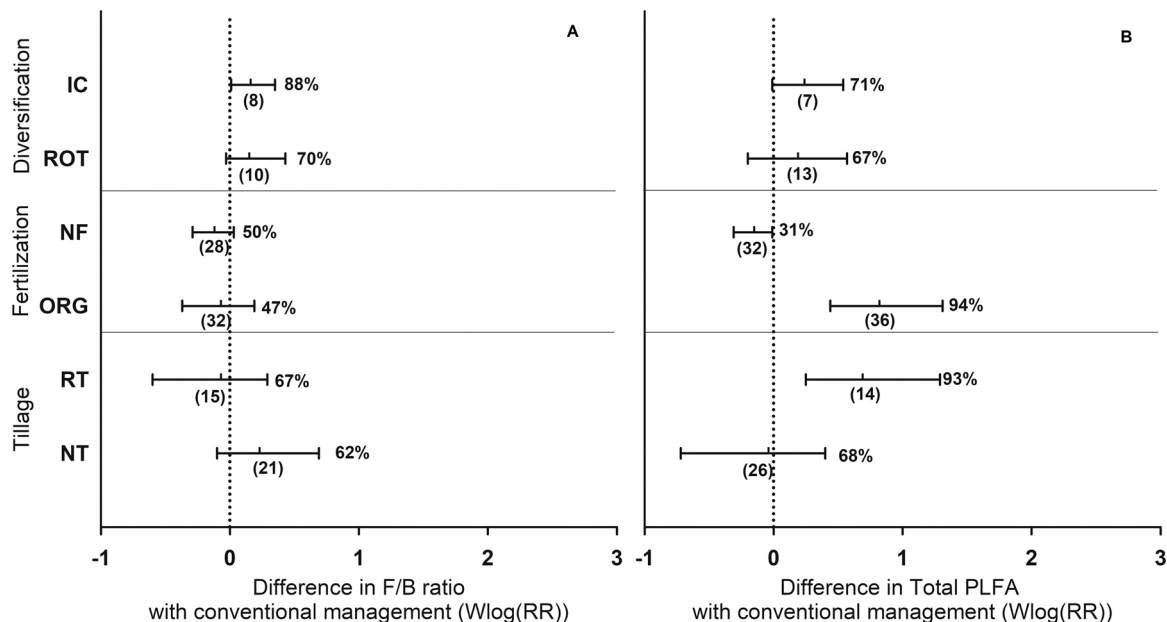

Fig. 4. Effect of diversification (IC: intercropping, ROT: rotations), fertilization (NF: no fertilization, ORG: organic fertilization) and conservation tillage (RT: reduced tillage, NT: no tillage) on fungal-to-bacterial PLFA ratio (A) and total PLFA (B). Values are shown as weighted log response ratio: Wlog(RR). The "I" denotes the mean and the horizontal bar represents the 95% confidence interval. The number below each bar indicates the size of the sample. Y-axis levels with means > 0 show that treatment increased the soil microbial property and decreased if means are < 0. The values in percentages indicate the fraction of studies showing positive effects of that factor on the studied property.

Supplementary material).

4. Discussion

4.1. General assessment on available microbiological data comparing sustainable management practices with conventional management in agricultural soils

This study has proven a lack of critical mass of research studies

dealing with soil metagenomics and next generation sequencing (NGS) comparing the effect of sustainable management practices in agriculture such as crop diversification, tillage intensity and fertilization type. Metagenomics methods have great potential to explore the abundance of specific microbial groups by use of qPCR and bacterial and fungal diversity by use of NGS and bioinformatics (Sangwan et al., 2016; White et al., 2017), not provided by PLFA analysis (Frostegård et al., 1993). New regulations, policies and strategies worldwide stand up for the use of sustainable management practices which enhance soil biodiversity (European Commission, 2020a, 2020b). However, scientific evidence is needed when selecting those practices which are more prone to foster increases in soil biodiversity and beneficial microbial groups, such as plant growth promoting bacteria. Thus, the scientific community has the duty to perform experiments that create knowledge about how management practices affect soil microbial community structure and biodiversity in agricultural lands, so that robust data is available for decision-makers and policymakers. The reach of a critical mass of studies dealing with the effect of sustainable management practices on soil microbial biomass, structure and biodiversity is essential to define patterns depending on cropping systems and pedo-climatic conditions, so that they can be integrated into regulations and policies.

PLFA analyses have been widely performed in agricultural soils to assess the effect of management since the 1990 s. The technique was initially developed and reported by White (1983), and has been accordingly used and generalized in the last decades, resulting in an effective methodology to assess microbial biomass and changes in microbial community structure (Frostegård et al., 1991, 1993; Frostegård and Bååth, 1996). However, metagenomics is more powerful to dig deeply into soil microbial diversity and microbial specific groups (White et al., 2017), which could provide indicators to properly assess the management practices needed to increase specific benefit organisms such as plant growth promoting bacteria. The lack of articles showing this type of information is due to the recent generalization in the use of soil metagenomics, NGS and associated bioinformatics, since this methodology is relatively new, and still progressing and improving.

4.2. Shifts in soil microbial abundances with the implementation of crop diversification, conservation tillage and organic fertilizers

Intercropping and rotations are beneficial for overall microorganisms, but the group most positively affected is fungi. Using both intercropping and rotations fosters and increases their abundance, higher than in bacterial abundance. This suggests that fungi seem more sensitive to changes in cropping systems than bacteria (Lazarova et al., 2019). Although most studies showed positive effects of crop diversification on total PLFA, F:B ratio and bacterial PLFA, the effect was not significant, because < 40% of the studies showed null or negative effects.

The adoption of no fertilization can have detrimental effects on microbial biomass, likely due to the exhaustion of nutrients needed for microbial growth and development (Wang et al., 2017). Nonetheless, using organic fertilizer instead of mineral fertilizers led to general increases in bacterial, fungal and total microbial biomass (Zhong et al., 2010). This is due to the provision of organic substrates that can be used by microorganisms as a source of energy and carbon (Gougoulias et al., 2014; Wang et al., 2017). Similar results were found by Bebber and Richards (2020) for soil microbial diversity. They showed that organic fertilization had a greater effect on functional and prokaryotic taxonomic diversity compared to mineral and non-fertilization, while no differences were found between fertilization and non-fertilization for fungal taxonomic diversity. Lori et al. (2017) found that organic inputs among other practices used in organic farming enhanced total microbial abundance and activity.

By far, together with the application of organic fertilizers, the implementation of reduced tillage had the highest positive effect on microbial, fungal and bacterial biomass. Similar results were found in a meta-analysis carried out by Li et al. (2020b) who showed that minimal

tillage enhanced microbial population size, bacterial diversity and fungal biomass. Contrarily, the adoption of no tillage showed no overall increase compared to conventional tillage in microbial abundance. However, Li et al. (2020a) found an increase in soil bacterial diversity under no tillage with no significant effect on fungal diversity. No tillage may cause problems of compactness and reduced porosity (Soto-Gómez et al., 2018; Ferreira et al., 2021), and soil sealing in some cases, with decreases in soil water content and gas exchange, or increases in water logging, especially long-term no tillage (Brady and Weil, 2002; Liu et al., 2020). These processes may limit the proper development of bacteria because of the lack of soil disturbance and breakdown of crop residue remaining on soil surfaces (Tyler, 2019). Nonetheless, this strategy was favorable for fungi, likely due to the lack of breakage of hyphae by tillage. Silvestro et al. (2018) showed that zero-tillage can modify fungal community structures promoting it, but also other factors such as season and sampling depth. Reduced tillage decreases frequency and/or length of tillage (Carter and McKyes, 2005), and so disturbance to soil microorganisms, mostly fungi, whose hyphae can be broken by tillage implements (Young and Ritz, 2000; Ritz and Young, 2004; Sharma-Poudyal et al., 2017). But compared to no tillage, it breaks the possible soil sealing and decreases compactness, favoring soil aeration, which can favor microbial activity and growth (Rincón-Flórez et al., 2020).

The lack of relationship between the response ratios of fungal, bacterial and total microbial biomass with soil physicochemical properties may suggest that the increases observed with the adoption of some management practices may be directly related to the adoption of that practice rather than to the shift in some physical or chemical soil property (Liua et al., 2017). In addition, those changes were generally not dependent on soil types with different values of pH, clay or soil organic matter. Microorganisms may respond to the organic substrates directly applied by the organic fertilizers, rather than the possible increases in soil organic carbon fostered by the application of this type of fertilizers (García-Orenes et al., 2016; Wanjala et al., 2019). Similarly, the degree of the response of the studied variables to crop diversification, fertilization type and tillage intensity was not controlled by mean annual temperature and precipitation, highlighting the overall benefits of sustainable management practices, mostly organic fertilization and reduced tillage on microbial biomass, independently of climate type (Morugán-Coronado et al., 2018; Zhang et al., 2020). So, although soil type and climate conditions control microbial abundance, the response of microorganisms to sustainable management compared to conventional managed is similar in different soils and climates (Pampulha and Oliveira, 2006). This fact is extremely important since it can help decision makers to propose and suggest sustainable management practices knowing that their adoption will be likely positive on microbial biomass regardless of the soil type and the climate.

4.3. Limitations and perspective

PLFAs are good indicators of microbial abundances which allow distinguishing between the main microbial groups, such as bacteria and fungi (Kaur et al., 2005; Frostegård et al., 2011), but provide limited information about microbial biodiversity, i.e., species variability at genotypic level (Zelles, 1999; Willers et al., 2015).

The effect of crop diversification, tillage or fertilization on other microbial parameters like microbial count, diversity, community structure, enzymatic activities and C-biomass were previously analyzed separately using meta-analysis. Thus, Zuber and Villamil (2016) studied the effects tilling had on microbial biomass carbon and enzyme activity, showing an overall increase of these variables in no-till compared to conventional tillage. Li et al. (2020a) showed clear benefits of no tillage on bacterial, fungal and actinomycete counts, but no clear results regarding the diversity index and microbial community structure. In relation with fertilization, Treseder (2008) showed a negative effect of nitrogen fertilization on microbial biomass, but not on bacterial or

fungal biomass. Bebber and Richards (2020) studied the effect of NPK and organic fertilization on taxonomic and functional diversity, showing no clear effects of those treatments on taxonomic diversity. However, NPK treatment increased functional diversity in relation with no fertilization and organic matter increased it over both no fertilization as well as NPK. McDaniel et al. (2014) studied the effect of crop diversification on microbial biomass, showing positive effects of this practice on this variable. Venter et al. (2016) showed increases in microbial richness and diversity when crop diversification increases. These studies focused only on one of the three mentioned cropping practices, and also are restricted to few microbial parameters with a restricted amount of data (generally <50). In order to have a more general overview, more studies including a high number of microbial parameters together and different management practices are needed to perform more robust meta-analyses and therefore better information for decision making.

Even though the current meta-analysis was performed with peer-reviewed studies published until July 2020, the majority of them were published in the last decade. However, those found providing information about metagenomics and molecular analysis had been published in the last three years. This indicates that new technology is being developed regarding metagenomics analysis on soil microorganisms. Also, high scientific interest is being brought up to deal with questions related not only with microbial abundance but also with providing new information on genus richness and diversity. Thus, there is a need for further studies dealing with soil microbial community structure and biodiversity by using metagenomics and NGS to define which management practices are most adequate to benefit soil microorganisms, mostly focusing on increasing soil biodiversity and benefit microorganisms that contribute to increase soil fertility, decrease the incidence of soil-borne diseases and promotes crop growth and production.

5. Conclusions

Only studies dealing with the extraction and measure of phospholipid fatty acids, providing indicators about soil microbial, fungal and bacterial biomass reached a critical mass to perform meta-analysis. There was a lack of a critical mass of studies dealing with soil metagenomics and next generation sequencing that provided results on the effects of sustainable management practices in agriculture on soil microbial biomass, community structure and biodiversity, impeding the meta-analysis for them. The current meta-analysis findings highlight the overall positive effect of diversification (rotations and intercropping), organic fertilization and reduced tillage on total PLFA, fungal PLFA and bacterial PLFA, which were generally increased in comparison to monocultures, use of inorganic fertilizers and conventional tillage. However, adopting a strategy of non-fertilization had negatively affected total PLFA, while it showed no significant effect on bacterial and fungal PLFA. Likewise, the effect of crop diversification and the adoption of no tillage was not significant for bacterial PLFA nor total PLFA, compared to monocultures and conventional tillage, respectively. The fungal-to-bacterial ratio was not affected by the factors studied except for the use of intercropping, which tends to increase the proportion of fungi over that of bacteria. Thus, as a general pattern, the adoption of sustainable management practices, mostly organic fertilization and reduced tillage, has overall positive effects on soil microbial biomass and fungal and bacterial abundance, with this effect being independent of soil physicochemical properties and climatic factors such as mean annual temperature and precipitation. This indicates the global positive effect independently of soil type and climate of the region.

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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2022.107867.

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