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Review paper

# Anthropogenic drivers of soil microbial communities and impacts on soil biological functions in agroecosystems



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#### ABSTRACT

Anthropogenic interventions play a key role in promoting positive feedback of soil-plant-environment interactions, but systematic reports on how anthropogenic activities influence soil physiochemical, microorganism-induced properties and soil health are still limited. Here, we assessed the impact of anthropogenic interventions, including crop diversification in rotations, soil physical disturbance, synthetic chemical inputs, and biofertilizer use on soil microbial community structure and function, and the consequential effects on agroecosystem productivity and environmental sustainability. Summarizing the results of over 160 medium- to long-term experiments from various soil-climatic zones across the globe in this review illustrated that (1) increasing crop diversification in rotations could bring positive impacts on soil microorganisms and soil health, especially including legume crops in rotations. (2) However, monocultures such as continuous wheat cropping could negatively impact soil health by enhancing activities of host specific pathogens. (3) Physical agronomic practices such as tillage can alter soil microbial communities by shifting microclimate conditions. (4) Mineral nitrogen fertilizer use, a leading nutrient input, may have exceeded the planetary boundary of N cycling, and is causing soil acidification and decreasing microbial biomass. (5) Synthetic chemicals, essential for disease management (pesticides) and yield sustainability (fertilization) in conventional agroecosystems are often toxic to non-target soil microorganisms, while bio-fungicides and biofertilizers-a more sustainable approach-carry significant risks to trigger succession of the native soil microbial community, thus impacting soil health. The key is to establish a rational balance between anthropogenic activities for agroecosystem productivity and potential negative influences on the soil microbial community and long-term soil health.

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# 1. Introduction

The global human population increased from 5.2 billion in 1990–7.7 billion in 2018 and is predicted to reach 9.7 billion by 2050 (Anonymous, 2019). The drastic increase in food demand associated with the ever-increasing human population raises concerns for global food security (Hodson, 2017), air, soil, and water pollution (Hou and Ok, 2019; Tollefson, 2019; Xu et al., 2019), and environmental sustainability (Puma, 2019; Tilman and Clark, 2014). An unanswered question remains: can the world meet food and fiber demands without damaging soil resources and the environment?

Anthropogenic activities play a significant role in regulating the production of sufficient amounts of nutritional food while preserving soil resources and the environment (Powers et al., 2016; Smith and Myers, 2018). Many crop-related farming practices have been developed to provide better food and fiber sources for humans, including 'crop rotations'—a common farming practice used since ancient Greek times (Hort, 1926)—to increase food production while maintaining soil fertility, 'break crops' to break the life cycle of pests while offering biological benefits to the soil (Barbieri et al., 2019; Li et al., 2018a), and 'crop diversification' to improve the judicious use of natural resources, while enhancing ecological resilience (Faostat, 2018). However, other than providing positive benefits to human society and the Earth, many of these anthropogenic activities are often over applied, which has dramatically altered natural landscapes (Fig. 1), changed the soil quality and harmed the environment as a consequence (Huang et al., 2018; Smith and Myers, 2018).

Soil quality largely depends on the physiochemical, biochemical, and biological properties of the soil. Soil microbial communities are particularly sensitive to anthropogenic activities (Wang et al., 2016). For example, excessive fertilization leads to negative effects on the environment including soil acidification, increased greenhouse gas emissions and P runoff causing eutrophication of waterbodies (Guo et al., 2010; Qu et al., 2014; Stevens, 2019). Conversely, crop diversification can alter the composition of soil microbial communities (Gurr et al., 2016; Thakur et al., 2015) and enhance ecosystem processes mediated by microbes, such as nutrient cycling (Cernay et al., 2016). Many anthropogenic interventions can cause shifts in the function of soil bacterial communities (Martyniuk et al., 2019). However, we do not fully understand how anthropogenic interventions



Fig. 1. Aerial photographs of farmland located in Western Canada showing how anthropogenic interventions in agroecosystems influence local landscape. (A) Google map showing how landscaping is modified by human activities from original grassland and valley area. (B) close-up of research field in 2011, (C) close-up in same research field in 2017, (D) same field in 2018 showing crop diversification management. Stars indicate landmarks. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

associated with medium- to long-term soil and crop management practices affects the diversity, structure and function of soil microbial communities.

Given the global context of food security, natural resource sustainability and environmental quality, anthropogenic interventions in agroecosystems must be carefully assessed against these consequences (Faostat, 2018). In particular, medium- to long-term anthropogenic interventions may negatively influence soil health and the functional diversity of soil microbial communities, which is the key indicator for soil health (Ryan et al., 2008), and gives rise to concerns about the consequences of intensified land use practices and excessive application of agrochemicals on human and environmental health (Monkiedje et al., 2006). This review summarizes the impact of anthropogenic activities, especially medium to long-term soil management and cropping practices, on soil health, with an emphasis on soil microorganisms (Fig. 2). This review aimed to examine the effect of various anthropogenic activities in agroecosystems on (1) the diversity and (2) functional activity of soil microbial communities, and (3) to reveal how soil microbial communities restructured related to long-term crop rotations. This information will aid policymakers, food producers and farmers in developing strategies to increase food production and quality while improving soil health and environmental sustainability.

# 2. Methods

A database was compiled to estimate the relationships among tested long-term anthropogenic activities and soil microorganisms on a global scale by searching databases of Canada Federal Science Library (FSL) in English (*https://fsl-bsf.scitech.gc.ca/ eng/intranet/home/*). For each section presented in this review paper, the particular agronomic term such as "rotation", "tillage", "disease control" etc. combined with "long-term" were included in the primary search. Then pre-selected references from the database were reorganized based on their publication years and impact factor (FI) of journals. These references were further divided into several sub-groups based on their experimental continents and locations. A total of 160 studies selected globally were included in the final database for this review. Some references included multiple sites, years, crops per study or agronomic treatments may be included in multiple sections. For each study, we also noted crop rotation series and crop cultivars included in rotations, soil type and GPS coordinates at experimental locations, experimental years and regions (Tables 1–4). Main effects of each reference were also summarized and listed in tables.

# 3. Selective effects of crop diversification on the diversity and functional activity of soil microbial communities

Temporal (crop rotation) and spatial (intercropping) diversification are important tools for increasing agricultural productivity and enhancing the sagacious use of natural resources (Barbieri et al., 2019). Optimizing crop diversity in both time and space can exploit soil resources such as water and nutrients and maximize the use of niches and soil biodiversity (Table 1). Root exudates from crops are important nutrients and signalling chemicals by which crops can alter bulk soil and rhizosphere microbial communities. Different crop species/cultivars can release a wide range of root exudates into fields which may have variable, long-term impacts on sustainability in agroecosystems (Gan et al., 2016). Crop plants can release up to 21% of their total fixed carbon as root exudates in the rhizosphere, which has a dramatic impact on soil microorganisms (Li et al., 2019). However, there are gaps in our understanding of how crop diversification can impact soil microorganisms and soil health in long-term rotations.

### 3.1. Legumes based cropping systems and their impacts on soil microorganisms

# 3.1.1. Biological nitrogen fixation and other soil nutrients changes caused by legume crops can modify soil microbial community composition

Legume crops extend available N pools in agroecosystems through biological nitrogen fixation (BNF) and other soil nutrients due to microbial activities associated with legume crops (Drinkwater et al., 1998). The total amount of global nitrogen fixation can reach over 400 Tg per year from both terrestrial and marine systems (Fowler et al., 2013), and in agriculture, BNF can contribute up to 70 Tg of available N annually (Herridge et al., 2008). In Western Canada, Gan et al. (2015) reported that adding legume crops, such as dry pea (Pisum sativum L.), lentil (Lens culinaris spp. culinaris) or chickpea (Cicer arietinum L.) in crop rotations increased N use efficiency, protein yield and total grain productivity by 33-51% in general. Furthermore, Hamel et al. (2018) reported a two-fold increase in soil N with two phases of pea in 4 year crop rotations. A study in India that incorporated mungbean in rotation reported significantly higher N (34%), P (46%), K (36%) and S (56%) uptake in the following crop, and incorporating chickpea in rotations significantly increased N (18%), P (19%), K (22%) and S (32%) uptake in the following crop, which was due to improved soil C and N nutrient conditions by including pulse crops in rotations (Venkatesh et al., 2017). A study in Australia showed that diversifying cropping systems by rotating legumes-peanut (Arachis hypogaea L.) and soybean (Glycine max L.)—with sugarcane (Saccharum officinarum L.) significantly reduced the abundance of ammonia oxidizers (24-44%) and amoA gene (30–35%) encoding ammonia monooxygenase which is the key enzyme of ammonia oxidization in the N cycle (Paungfoo-Lonhienne et al., 2017). These results highlight the potential influence that legume crops can have on soil nutrient availability in rotations, which appears to be linked to the modification of the functional activity of the soil microbial community. In a Canadian study, including three legume phases in long-term rotations can lead to higher soil nitrogen potential in agroecosystems and significantly changed the composition and function of the rhizobacterial community compared with a wheat monoculture (Hamel et al., 2018). Different legume species in a crop rotation can impact the diversity and structure of





Table 1Crop diversification impact	s on soil microorganisms in the	long-term.		
Original paper	Rotation series	Soil type and GPS coordinates	Rotation year and region	Effects
North America (Lay et al., 2018)	Canola-legume and canola-cereal vs. continuous canola	Lacombe (52°28'N - 113°44'W) and Brandon (49°50'N - 99°57'W) are located in Black Chernozemic soil zone, while Beaverlodge (55°12'N - 119°25'W) is located in Dark Gray	2008–2014: Lacombe, Brandon and Beaverlodge, Canada	Core microbial community positively correlated with canola yield with crop diversification
(Paré et al., 2015) (McGill et al., 1986)	Canola-pea-barley vs. continuous pea Wheat-fallow vs. wheat-barley-forage-fora- or-fallow	son zone Eluviated Gleysolic silty clay (48°25'N - 71°03'W) Dark Gray Luvisol (53°7'N - 114°29'W)	1999–2010; Saguenay–Lac- Saint-Jean, Canada 1930–1982; Brenton, Canada	Soil nutrients increased with crop diversification, but canola-included rotations had lower P and SOC in topsoil layer than continuous pea Higher diversification increased soil microbial biomass N by 117%
(Bernard et al., 2012)	out-clover-buckwheat ± r- apeseed (green manure)-potato vs. barley ± rapeseed (green manure)-notato	Mapleton loam and Caribou gravelly loam (45°8'N – 69°13'W)	Over 35 years before sampling in 2007; Two farms located in Maine, USA	Richness and diversity of bacterial community higher with rapeseed in rotation; shift in functional bacterial groups using different carbon resources with rapeseed in rotation
(Martyniuk and Wagner, 1978)	Continuous wheat/corn vs. corn-oat-wheat-red clover	Alfisol (38°17′N – 92°29′W)	1888–1976; Missouri, USA	Increased crop diversification added extra organic carbon, considerable fixed nitrogen, and higher pH which could explain higher amounts of hacteria and some funoi
(Robert et al., 2010)	Potato rotated with several crops	Nokomis sandy loam (44°38'N – 124°3'W)	1997–2006; Newport, USA	Canola significantly restricted fungal pathogen related to potato after 7- Canola significantly restricted fungal pathogen related to potato after 7- year rotation; in particular, three common potato tuber diseases-Rhizotronia canker, black scurf and common scab-declined by Japont 407, 388, and 708, resciencinely, after canola
(Collins et al., 1992)	Wheat-fallow/pea vs. continuous wheat and grass pasture	Walla silt loam (45°40'N – 118°47'W)	1931–1987; Pendleton, USA	by about they are always respectively, and values a property of including pea in rotation increased soil microbial biomass carbon and nitrogen by up to 421 and 79 mg kg <sup>-1</sup> , respectively, after 57-year rotation, and increased the diversity of functional bacterial subgroups that are involved in $N - A - C = \frac{1}{2}$ .
(Dick, 1984)	Continuous corn vs. corn-soybean and corn-oar-alfalfa	Silty loam (40°22'N – 82°59'W)	1962–1980; Ohio, USA	that are important in the and expering Increased crop diversification (legume involved) enhanced soil- microbial-based enzymes in the long-term
(Peralta et al., 2018)	Continuous corn vs. Continuous corn vs. corn+red clover (as cover crop)/soybean (-wheat/wheat+red clover (	Loamy soil (44°10′N – 84°30′W)	2000–2012; Michigan, USA	In the same rotation, adding cover crop increased bacterial diversity and richness; crop diversification enhanced <i>pmD</i> gene, which is related to soil-borne disease suppression
(Howard et al., 2017)	Continuous corn vs. Continuous corn vs. corn-winter legume and cotton+winter wheat-corn+winter legume-soybean	Soil type not reported (47°18'N – 122°13'W)	1896-2012; Auburn, USA	Soil microbial enzymes—acid phosphatase, alkaline phosphatase and phosphodiesterase—increased with crop diversification, especially under non-irrigation conditions
				(continued on next page)

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Table 1 (continued)				
Original paper	Rotation series	Soil type and GPS coordinates	Rotation year and region	Effects
Europe (Cwalina-Ambroziak et al., 2016)	Canola-wheat-pea vs. continuous canola	Loess soil (53°41'N – 19°57'E)	2009–2013; Bałcyny, Poland	Including legume and cereal in rotation reduced many pathogenic soil microbes, but increased <i>Botrytis cinerea</i> , with infection index on canola leaves as 1.7% in rotation and 0.8% in monoculture
(Gałązka et al., 2017)	Continuous maize vs. spring barlev-winter wheat-maize	Soil type not reported (52°20'N – $21^{\circ}6'E$ )	2004–2012; Mazowieckie Voivodeshin. Poland	Increased crop diversification decreased total numbers of oligotrophic and conjorrophic bacteria compared with continuous maize
(Hegewald et al., 2017)	Canola-wheat vs.	Haplic chernozem (51°57'N – 11°41'E)	2005–2014; Saxony-Anhalt, Germany	Continuous canola had 72% higher blackleg incidence caused by fungal
(Babin et al., 2019)	Maize-wheat-barley-rape- seed-wheat	Loess chernozem (51°47'N – 11°43'E)	Germany Germany	Wheat fail after rapeseed had higher abundance of <i>Chitinophaga</i> , <i>Ktedonobacter</i> , <i>Phyllobacterium</i> , and <i>Massilia</i> , while <i>Anderseniella</i> and <i>Communus</i> were only found in wheat after maize
(Laudicina et al., 2015)	Wheat-bean vs. continuous wheat	Xeric Chromic Haploxerert (37°30'N – 13°31'E)	1991–2009; Sicily, Italy	including bean in rotation significantly increased ratio between soil microbial biomass carbon and rotal soil carbon
(Le Guillou et al., 2019) Acia	Multiple legume involved rotations	Silty loam (48°00'N – 2°49'E)	1993–2013; Brittany, France	Including legume crops into rotation increased soil microbial biomass
(Parihar et al., 2016)	Maize-wheat/chickpea/ mustard/maize-mungbean/ Sechania	Sandy loam (28°40'N - 77°12'E)	2008–2014; New Delhi, India	With chickpea in rotation, MBC, microbial activity and activities of dehydrogenase, β-glucosidase and alkaline phosphatase increased more than other rotations
(Maarastawi et al., 2018)	Continuous rice and continuous maize vs. rice-maize	Silty clay for Los Banos (14°11′N – 121°15′E) and silty loam for Tarlac (15°32′N – 120°37′E)	Over 20 years for monocultures and 4 years for rotation; Los Banos and Tarlar Philinnines	Crop rotation induced specific microbial responses in the bulk soil and rhizosphere of the cultivated crop
(Lu et al., 2018)	Rice-rice-fallow vs. rice-rice-rapeseed	Red soil (26°79'N – 113°19'E)	1985–2016; Hunan, China	Soil enzymes, such as catalase, acid phosphomonoesterase and cellulose, increased up to three times and soil microbial N, N and P increased with rapeseed in rotation compared with fallow
South America (Balota et al., 2004)	Soybean-wheat, maize-wheat, cotton-wheat	Oxisol (23°40'S – 50°52'W)	1976–1997; Paraná, Brazil	Maize–wheat rotation had highest activities of amylase, cellulose, arylsulfatase, acid phosphatase and alkaline phosphatase (>10%, >9%, >25%, >6% and >10% higher than the other two rotations, respectively)

Table 2Long-term rotation involved tills	ge effects on soil microorganism	š			
Original paper	Rotation series	Tillage	Soil type and GPS coordinates	Rotation year and region	Effects
North America (Lupwayi et al., 2017)	Multiple rotation series	Conventional tillage vs. reduced tillage	Dark Brown Chernozem (Typic Borroll in Soil Taxonomy; Haplic Kastanozem in World Reference Base (50°03'N – 112°09'W)	2000–2011; Vauxhall, Canada	Reduced tillage significantly increased soil microbial biomass carbon from 428 to 509 mg C kg <sup>-1</sup> and increased fungal and bacterial PLFAs in both bulk and rhizosphere soil when compared with conventional
(Schmidt et al., 2018)	Tomato-cotton	No-tillage vs. conventional tillage	Panoche clay loam (36°20'N – 120°7'W)	1999–2013; California, USA	tillage Compared to no-till, tillage reduced the number of bacteria and archaea by around 4×10 <sup>7</sup> and 1.5×10 <sup>7</sup> in the top 5-cm soil
(Wang et al., 2017)	Multiple rotating series were applied	No-tillage vs. conventional tillage	Delanco fine sandy loam (35°25′N – 82°33′W)	1994–2009; North Carolina, USA	Layer No-till increased total PLFA concentration by 55% and bacterial PLFA by 22% compared to
(Chuntao et al., 2017)	In Washington, winter wheat-spring wheat for no- till, winter wheat-fallow for tillage In Idaho, winter wheat-spring wheat for wheat-spring model for no-till	No-tillage vs. conventional tillage	Palouse silty loam (38°54'N - 77°2'W for Washington, and 45°29'N - 115°27'W for Idaho)	35 years before first sampling in 2008 in Washington, and 2000-2010 in Idaho, Washington and Idaho, USA	Current and the significantly influence soil bacterial diversity, but strongly impacted bacterial community composition (Chitinophagaceae, Micrococcaceae, Gaiellaceae and Nocardioidaceae more frequent under tillage, while Hyphomicrobiaceae, acidobacteriaceae dominated under no-till)
(Wyngaard et al., 2016)	Cotton/com-rye/wheat (1994-2005)-canola-pearl millet/rye (2006-2010)-fallow (2011-2013)	No-tillage vs. conventional tillage	Cecil series soil (33°52'N – 83°27'W)	1994–2013; Watkinsville, USA	Convertional tillage significantly reduced the Convertional tillage significantly reduced the amounts of ammonia-oxidizing bacteria (AOB) in the and ammonia-oxidizing bacteria (AOB) in the top 20 cm soil layer, which could slow down the ortential of N mineralization
(Somenahally et al., 2018)	Wheat-legume	No-tillage vs. conventional tillage	Brewer silty clay loam soil (35°57'N – 98°03'W)	2011–2017; El Reno, USA	Fungal biomass carbon under tillage was 193% lower with added inorganic N but 26% higher with added organic N, compared with no-till
Ludicina et al., 2015) (Laudicina et al., 2019) (Babin et al., 2019)	Wheat-bean vs. continuous wheat Maize-wheat-barley-rape- seed-wheat	No-tillage vs. conventional tillage vs. reduced tillage Conventional tillage vs. mouldboard plow tillage	Xeric Chromic Haploxerert (37°30'N – 13°31'E) Loess chernozem over limestone (51°47'N – 11°43'E)	1991–2009; Sicily, Italy 1992–2015; Bernburg, Germany	Conventional tillage had lowest total organic C, microbial biomass C and respiration rate Conventional tillage had more genera belonging to Alphaproteobacteria and Actinobacteria, but fewer Acidobacterial order
(Sommermann et al., 2018)	Maize-wheat-barley-rape- seed-wheat	Conventional tillage vs. mouldboard plow tillage	Loess chernozem over limestone (51°47N – 11°43E)	1992–2015; Bernburg, Germany	Gp4 and Sphingomonas Conventional tillage selected fungi, such as Stagonospora, Claroideoglomus, and Rhizophagus, in wheat rhizosphere, while Entrophospora and Sebacina

(continued on next page)

Table 2 (continued)					
Original paper	Rotation series	Tillage	Soil type and GPS coordinates	Rotation year and region	Effects
(Le Guillou et al., 2019)	Multiple legume involved rotations	No-tillage vs. conventional tillage	Silty loam (48°00'N - 2°49'E)	1993–2013; Brittany, France	Tillage significantly reduced soil microbial biomass and fungal richness by at least 2.4%, but increased bacterial richness and evenness
(Henneron et al., 2015)	Wheat-pea-wheat-oilseed/ maize	Conventional tillage vs. conservation tillage	Luvisol (48°48'N – 2°08'E)	1997–2010; Versailles, France	compared to no-tillage Conservation tillage increased soil bacteria and fungi biomass and abundance of $\alpha$ - <i>Proteobacteria</i> and <i>Actinobacteria</i> compared to
(Gałązka et al., 2017)	Continuous maize vs. spring barley-winter wheat-maize	No-tillage vs. full plowing tillage	Soil type not reported (52°20'N – 21°6'E)	2004-2012; Mazowieckie Voivodeship, Poland	conventional tillage Highest numbers of oligotrophic and copiotrophic bacteria after harvest under no- tillage
Asia (Essel et al., 2018)	Pea-wheat	No-tillage vs. conventional tillage	Calcaric Cambisol (35°28'N – 104°44'E)	2001–2016; Dingxi, China	No-tillage increased fungal and bacterial species diversity through improved soil chemical properties, especially with stubble
(Li et al., 2015)	Rapeseed-rice	Ridge with no-tillage vs. conventional tillage	Hydragric Anthrosol (30°26'N – 106°26'E)	1990–2012; Chongqin, China	residue in ricid armoA gene from AOB and AOB procedure was 50% and 29% higher under ridge with no-
(Yang et al., 2016)	Wheat-rice	Shallow tillage vs. shallow tillage with ditch-buried straw return	Gleyi-stagnic anthrosol (32°13'N – 120°63'W)	2008-2014; Nantong, China	tunage Shallow tillage with ditch-buried straw return in 20-cm soil layer increased peroxidase, cellobiohydrolase, urease, and acid phosphatase activities by 3.5%, 75.0%, 81.4% and 41.7%, respectively
South America (Balota et al., 2004)	Soybean-wheat, maize-wheat, cotton-wheat	No-tillage vs. conventional tillage	Oxisol (23º40'S – 50°52'W)	1976–1997; Paraná, Brazil	Higher activities of amylase, cellulose, arylsulfatase, acid phosphatase and alkaline phosphatase activities under no-tillage (37%, 37%, 215%, 8% and 47% higher than
(Hungrià et al., 2009)	Soybean-wheat, soybean-wheat-lupin-mai- ze-oat-radish	No-tillage vs. conventional tillage vs. field cultivator tillage	Oxisol (23°11'S – 51°11'W)	1996–2008; Paraná, Brazil	conventional tillage, respectively) Highest soil microbial biomass C and N under no-tillage (up to 607 and 89 mg kg <sup>-1</sup> dry soil, respectively) compared with conventional and
(dos Santos et al., 2016)	Soybean-fallow vs. soybean-sorghum-fallow vs. maize-pigeon pea-fallow	No-tillage vs. conventional tillage	Clayey Oxisol (15°33'S – 47°44'W)	1994–2013; Embrapa Cerrados, Brazil	neut cutivator tinage Conventional tilage significantly modified denitrifier community and increased cumulative N <sub>2</sub> O (up to 1.35 kg ha <sup>-1</sup> ) compared to no-tillage

ilization effects on soil microo	rganisms.			
Rotation series	Fertilization	Soil type and GPS coordinates	Rotation year and region	Effects
Wheat-legume	No N vs. inorganic N vs. organic N	Brewer silty clay loam (35°57'N – 98°03'W)	2011–2017; El Reno, USA	Fungal biomass carbon 193% higher with added inorganic N but 26% lower with added organic N compared with no N; gene copies of denitrifiers at least 20% higher in inorganic N
No details provided	Organic fertilizer vs. NPK	Delanco fine sandy loam (35°25′N – 82°33′W)	1994–2009; North Carolina, USA	treatment than others Organic fertilizer increased total PLFA concentration by 28% and bacterial PLFA by 23%
Continuous corn vs. corn-soybean vs. corn-oats-alfalfa	No fertilization vs. NPK vs. manure	Soil type not reported (39°44'N – 89°27'W)	1876–2009; Illinois, USA	Compared to chemical retunct Higher bacterial diversity and species richness Higher manure than other two treatments; in particular, OTUs of <i>Acidobacteria</i> and <i>Proteobacteria</i> in NPK were 200% and 50%
Wheat-fallow vs. wheat-barley-forage-fora-	No fertilization vs. manure vs. NPKS	Dark gray Luvisol (53°7'N – 114°29'W)	1930–1982; Brenton, Canada	lower than other two treatments Manure treatment contained up to two times more microbial N than NPKS and no
ge-1a110w Flax-alfafa-alfafa-wheat	No fertilization vs. NPK	Humic Vertisol with heavy clay (49°38'N – 97°8'W)	1992–2011; Winnipeg, Canada	Perturbation treatments P-cycle-related <i>phoD</i> gene analysis showed 26 unique OTUs related to no fertilization treatment, and 16 unique OTUs related to mineral fertilization
Maize-field pea	No fertilization vs. NPK	Soil type not reported (25°45'N - 28°16'E)	1939–1998; Pretoria, Africa	Compared to no fertilization, NPK fertilization significantly increased soil microbial biomass C and N, and the number of bacteria, actinomycetes and fungi

Long-term rotation related fertiliza Table 3

Original paper

(Somenahally et al., 2018)

North America

(Soman et al., 2017)

(Wang et al., 2017)

(McGill et al., 1986)

(Fraser et al., 2015)

10

Africa (Belay et al., 2002)

Asia

(continued on next page)

intensive fertilization, whereas Diversispora and Funneliformis associated with extensive application Ambispora and Paraglomus correlated to

Higher N fertilization increased soil bacterial abundance

1992-2015; Bernburg,

Loess chernozem over

 $220\,kg\,ha^{-1}$  N vs. 90 kg ha^{-1} N

Maize-wheat-barley-rape-

(Babin et al., 2019)

Europe

seed-wheat

 $220~kg\,ha^{-1}$  N vs. 90 kg  $ha^{-1}$  N

Maize-wheat-barley-rape-seed-wheat

et al., 2018) (Sommermann

limestone

Germany

1992-2015; Bernburg,

Germany

Loess chernozem over limestone (51°47'N - 11°43'E)

(51°47'N - 11°43'E)

Table 3 (continued)					
Original paper	Rotation series	Fertilization	Soil type and GPS coordinates	Rotation year and region	Effects
(Gong et al., 2009)	Winter wheat-summer maize	No fertilization vs. NPK vs. manure	Aquic Inceptisol (35°00'N – 114°24'E)	1989–2007; Henan, China	Number of total bacteria, fungi and actinomycetes increased by at least 50% with either organic matter or chemical fertilizer invurt combared with no fertilization
(Qin et al., 2015)	Wheat-rice	No fertilization vs. straw vs. manure vs. NPK vs. NPKS vs. NPK+manure	Silty clay loam (30°09'N - 120°06'E)	1996–2013; Zhejiang, China	Figure Compared with the change AMF colonization Fertilization did not change AMF colonization rate, but increased the amount of AMF fatty acid indictors; the composition of AMF community changed with fertilization
(Zhang et al., 2016b)	Wheat-maize	No fertilization vs. NPK vs. NPK+manure vs. NPK+straw	Calcaric cambisol (35°00'N – 113°41'E)	1990–2013; Yuanyang, China	Compared to no fertilization, fertilization significantly increased microbial biomass C and N by at least 100 and 5.5 mg kg <sup>-1</sup> , respectively, in the wheat phase, and 60 and 30 mg kg <sup>-1</sup> respectively in the maize ohase
(Ai et al., 2018)	Wheat-maize (1990–2012) then split into wheat-maize and wheat-soybean from 2013 to 2016	No fertilization vs. NPK vs. NPK+manure	Aquic Ustochrept (34°28'N – 112°30'E)	1990–2016; Zhengzhou, China	Fortilization increased copiotrophic taxa Fertilization increased copiotrophic taxa Affiliated with <i>Pseudomonadacee</i> and Cytophagaceae bacterial families, but reduced some Acidoharteria such as sulvaround R1841
(Buragohain et al., 2018)	Rice-fallow	No fertilization (T1) vs. 100% recommend doses of inorganic NPK (T2) vs. 50% inorganic N and P+100% K+ biofertilizer (T3) vs. 50% inorganic N and P+100% K+1 t ha <sup>-1</sup> enriched biocompost (T4) vs. 75% inorganic N and P+100% K+2 t ha <sup>-1</sup> enriched biocompost (T5)	Aeric Endoaquept (26°43'N – 94°11'E)	2006–2015; Assam, India	Increased the amount of most tested soil enzymes, including dehydrogenase, phosphatase and urease, and microbial biomass carbon and the number of Azospirillum and Bacillus spp. in T4 and T5

#### Table 4

Long-term rotation related anthropogenic activities effects on greenhouse gases.

Original paper	Rotation series and related activities	Soil type and GPS coordinates	Rotation year and region	Effects
North America				
(Behnke et al., 2018)	Continuous corn vs. corn +soybean vs. corn+soybean +wheat vs. soybean+corn vs. continuous soybean; tillage vs. no-till	Silty clay loam (40°55′N - 90°43′W)	1996–2015; Illinois, USA	Soybean in rotation reduced daily and cumulative greenhouse gas (GHG) emissions, especially N <sub>2</sub> O; soybean-corn had much lower GHG emissions than corn-soybean; rotation combined with tillage significantly increased daily CO <sub>2</sub> emissions but not cumulative GHG emissions
(Ussiri and Lal, 2009)	Corn–soybean (3 cycles) then continuous corn; no-till vs. reduced tillage vs. moldboard plow tillage	Crosby silty loam (39°45′N – 83°36′W)	1956–2005; Ohio, USA	Long-term no-tillage combined with rotations reduced $CO_2$ emissions by an average of 0.65 Mg C ha <sup>-1</sup> yr <sup>-1</sup> compared to tillage treatments
(Ozlu and Kumar, 2018)	Corn-soybean; low, medium and high manure treatments vs. medium and high inorganic fertilizer treatments vs. no fertilization	Vienna soil (44° 22′N – 96° 47′W)	2008–2016; South Dakota, USA	Fertilization in rotations increased GHG emissions, especially under inorganic fertilizer treatment; manure application lead to higher global warming potential rates than other treatments
(Jarecki et al., 2018)	Continuous corn vs. corn-corn-soybean-soybean vs. corn-corn-soybean-wheat vs. corn-corn-soybean-wheat with red clover as cover crop vs. corn-corn-alfalfa-alfalfa; conventional tillage for all plots	Elora: Gray Brown Luvisol (43°39'N – 80°25'W) Woodslee: Brookston clay loam (42°13'N – 82°44'W)	Elora 1981–2015; Woodslee 1959–2015; Elora and Woodslee, Canada	Crop diversification in rotations increased observed and predicted soil C sink capabilities
(Ma et al., 2012)	Soybean-maize vs. alfal/ clover-maize vs. continuous maize; four levels of N treatments (0, 50, 100, and 150 kg N ha <sup>-1</sup> ) in rotational maize phase, and six levels (0, 50, 100, 150, 200, and 250 kg N ha <sup>-1</sup> ) in the continuous maize phase	Soil type not reported (45°22'N – 75°43'W)	1992–2010; Ottawa, Canada	Maize production with 100 kg N ha <sup>-1</sup> in rotation after forage or grain legumes maintained high productivity with low GHG emissions and C footprint, relative to continuous maize cropping with 200 kg N ha <sup>-1</sup>
South America (Bayer et al., 2015)	Black oat-maize vs. vetch-maize vs. oat-vetch/ maize-cowpea; 0 vs. 180 kg ha <sup>-1</sup> N	Alumic Acrisol (30°06′S – 51°4′W)	1985–2004; Eldorado do Sul, Brazil	Yield-scaled $N_2O$ emissions after vetch residue (67 g N Mg <sup>-1</sup> grain) were half that after urea-N (152 g N Mg <sup>-1</sup> grain)
(Salton et al., 2014)	Soybean-oat with tillage vs. soybean/corn-wheat/oat/turnip with no-tillage vs. soybean/ oat-pasture grass with no- tillage vs. permanent pasture grass	Oxisol (22°16′S – 54°48′W)	1995–2010; Dourados, Brazil	Soybean/oat-pasture grass with no-tillage very efficient at accumulating soil C and reducing GHG emissions, relative to other treatments under local environmental conditions
Asia	0			
(Cai et al., 2018)	Rice-wheat vs. rice-rapeseed vs. rice-milk vetch vs. rice-fava bean	Gleyi-Stagnic Anthrosol (31°01'N – 119°17'E)	2011–2016; Changshu, China	Legume included rotations reduced annual synthetic N inputs up to 56% compared to rotations without legumes, leading to consistent reductions in reactive N losses. Estimated C footprint 37–50% lower in rotations with legumes included than those without legumes
(Zhang et al., 2018)	Wheat-maize; no fertilization vs. NPK vs. NPK+swine manure vs. NPK+plus swine manure	Ferralic Cambisol (28°37'N – 116°26'E)	1990–2017; Changping, China	Cumulative NH <sub>3</sub> -N increased almost 10 and 18 times in rotation under NPK and NPK+plus swine manure treatments, respectively, when compared with no fertilization; cumulative GHG emissions of N <sub>2</sub> O-N and CO <sub>2</sub> -C increased 1.6 and 1.7 times, respectively, under NPK treatment and 3.8 and 3 times, respectively, under NPK+plus swine manure, relative to no fertilization; global warming potential increased almost 1.7 and 3 times under NPK and NPK+plus swine manure treatments, respectively, when compared with control
(Zhang, 2017)	Wheat-fallow; no fertilization vs. N vs. NP vs. manure vs. NP + manure	Caliche soil (35°13′N – 107°40′E)	1984–2009; Changwu, China	Long-term fertilization in rotations reduced root stubble C by 34% under N treatment, but increased root stubble C by 15–63% in the NP, manure, and NP+manure treatments; fertilization decreased root stubble C:N by 8–38%, which further influenced soil C sink capacities

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#### Table 4 (continued)

Original paper	Rotation series and related activities	Soil type and GPS coordinates	Rotation year and region	Effects
(Parihar et al., 2018)	Maize-wheat-mungbean vs. maize-chickpea-sesbania vs. maize-mustard-mungbean vs. maize-maize-sesbania; no- tillage vs. conventional tillage vs. no tilled permanent raised bed	Sandy loam (28°40'N - 77°12'E)	2008–2013; New Delhi, India	Global warming potential index up to 18% higher under conventional tillage than other two tillage treatments with all rotation treatments, and up to 11% higher under maize-maize-sesbania than other rotations
Europe	Continuous wheat vs. faba	Chromic	1991_2015	Potential denitrifying enzyme activities
al., 2018)	bean-wheat; no-tillage vs.	Haploxerert	Agrigento, Italy	abundance of <i>nosZ</i> gene and carbon pool stock
(Guardia et al., 2016)	Cereal-legume-fallow (1994-2004), then from 2005 starts to use fallow-wheat-vetch-barley; no-tillage vs. conventional	(37 30 N - 13 31 E) Calcic Haploxeralf (40°32′N - 3°20′W)	1994–2013; Madrid, Spain	Net global warming potential increased about 3.5 times in barley stage than vetch stage in rotation, but vetch produced higher global warming potential than barley under no-tillage
(Constantin et al., 2010)	Wheat-barley-pea in Boigneville; wheat-maize in Kerlavic; pea-wheat-sugar beet (1990-2003), then wheat-barley-sugar beet after 2003 in Thibie; no-tillage vs. conventional tillage; N and reduced N	Boigneville: Haplic luvisol (48°19'N – 2°23'E) Kerlavic: Dystric cambisol (48°03'N – 4°04'W) Thibie: Rendzina (48°56'N – 4°14'E)	1990–2008; Boigneville, Kerlavic and Thibie in France	Rotation was the most efficient technique to reduce N leaching (from $36\%$ to $62\%$ ) and remained efficient in the long-term; no-tillage and reduced N in rotations had a positive but smaller impact; N storage in soil organic matter markedly increased in rotation (by $10-24 \text{ kg ha}^{-1} \text{ yr}^{-1}$ )
(Henryson et al., 2019)	Barley–oilseed rape–wheat–beet at Southern sites; barley–oats–oilseed rape–wheat–oats at Central sites; three levels of N (low, medium and high, 50, 100 and 150 kg ha <sup>-1</sup> at Southern sites, and 40, 80 and 120 kg ha <sup>-1</sup> at Central sites)	Not reported	1998–2010; Multiple locations in Sweden	Long-term N fertilization in rotations had limited impact on GHG emissions (10%) and marine eutrophication (20%) between low and high inputs; GHG emissions at the Southern and Central sites were up to 280%, and marine eutrophication was up to 340%, suggesting site-specific information must be considered for rotation design and related agronomic activities to reduce GHG levels
(Hobley et al., 2018)	Clover/fava bean/ maize-wheat-rye-barley; no fertilization vs. PK vs. NPK	Eutric Fluvic Gleyic Cambisol (50°36'N – 8°39'E)	1982–2016; Gießen, Germany	Over 30 years of green mulching added an average of 4.1% C per annum to the soil profile compared with conventional crop rotation and fertilization
(Herold et al., 2012)	Wheat-potato-barley-swede- s-oat-grass-grass-grass	Iron podsol (57°8'N – 2°5'W)	1961–2009; Aberdeen, UK	Denitrification and potential N <sub>2</sub> O emissions influenced by crop diversification during long-term rotation
Oceania				
(Ma et al., 2018)	Chickpea-wheat-barley vs. canola-wheat-barley	Black vertisol (31°15′S – 150°98′E)	2021-2100 scenarios based on data collected from 2009 to 2011; New South Wales, Australia	N <sub>2</sub> O emissions increased by 35–44% for chickpea–wheat–barley and 72–76% for canola–wheat–barley; yield-scaled N <sub>2</sub> O-N emissions increased by 24–42% for chickpea–wheat–barley and 46–54% for canola–wheat–barley under two climate scenarios by the end of 2100
(Chauhan et al., 2015)	Maize-peanut vs. peanut-maize	Kandosolic Redoxic Hydrosol (14°27'S – 132°15'E)	2010–2050 scenarios based on data collected from 1960 to 2010; Katherine Australia	Irrigation requirement increased by up to 11% in peanut-maize but had smaller reductions in total soil organic C accumulation and smaller increases in N losses and GHG emissions than maize-neanut

soil microbial communities differently, and further modify their functions in soil. For example, the abundance of *Proteobacteria, Actinobacteria* and *Gemmatimonadetes* in soil varied with different legume crops, and these effects carried over to the following crop in the rotation (Yang et al., 2013). Fungal communities are also impacted by the inclusion of legumes in rotations, particularly in the phase following the legume crop. For example, while the arbuscular mycorrhizal fungal (AMF) community was not significantly modified during the legume phase, *Glomus* and *Funneliformis* became the dominant AMF genera in wheat roots after pea, and *Rhizophagus* was the dominant AMF genus in wheat roots after lentil and chickpea (Borrell et al., 2017), revealing the modification effects of legume crops on specific fungal groups. <u>Esmaeili Taheri et al.</u> (2016) reported higher yields of durum wheat after pea than after chickpea, which was attributed to the selective effects of these legume crops on fungi. In particular, the abundance of endophytic fungal antagonists doubled in wheat roots after pea relative to wheat after chickpea, and culturable virulent fungal pathogens in durum wheat root tissue were almost twice as much in wheat after chickpea than after pea,

suggesting a strong crop influence of shifting functional fungal communities on following crops. Integrating many legume crops in rotations can also build up host specific pathogens which can further impact crop yield and soil health (Bainard et al., 2017).

# 3.1.2. Legume crop root exudations can modify soil microbial community and selectively affect soil pathogen growth

Crop diversification with legumes alters soil biochemistry which can result in a restructuring of the soil microbial community. Legume roots can produce a variety of bioactive proteins that can selectively influence the soil fungal community (Bazghaleh et al., 2016). These include low-molecular-mass phytochemicals in legume roots that can selectively influence the fungal community by stimulating or inhibiting certain soil-borne fungal pathogens and fungal endophytes. In a study in Saskatchewan, Canada, chickpea genotypes were found to shape the soil microbiome and affect the establishment of the subsequent durum wheat crop (Ellouze et al., 2013). The impact of chickpea on durum wheat grown the following year in a rotation was enhanced by inoculation of root endophytes isolated from the same soil (Ellouze et al., 2015). A relationship between chickpea genotype and root phytochemical composition was related to the cultivar-specific effect on root endophytes (Ellouze et al., 2012). Therefore, root phytochemicals produced by pathogen-resistant legume crops could stimulate specific fungal species while inhibiting others; these effects are often cultivar specific. For example, chickpea cultivars that can synthesize trans-2-hexen-1-ol and 1-hexanol in their tissue had significant inhibition effects on Fusarium inoculum relative to those cultivars without this capacity (Cruz et al., 2012). In addition, Parihar et al. (2016) found incorporating legume phases in rotations increased soil enzyme activities—dehydrogenase,  $\beta$ -glucosidase and alkaline phosphatase—by up to 38%, 26% and 33%, respectively. These results partly explain the 'rotational effect' of legume-based diversification and how an optimized rotation design with specific legume species or cultivars can maximize the benefits for agroecosystems by increasing biological nitrogen fixation, reducing agrochemical use and breaking the life cycles of host-specific pathogens. However, careful consideration of crop diversification with legumes is important for capturing key long-term benefits for healthy soil because there are some potential risks associated with adding legume crops to rotations. Improper use of legume crops in rotation could negatively impact the structure of the soil microbial community and significantly accumulate pulse-specific pathogens which can cause serious disease such as root rot and lead to crop yield loss as a consequence. For example, continuous pea cropping has been shown to inhibit soil microbial activity, as the amount of total soil microbial PLFA biomarkers, dehydrogenase, phosphatase, urease, and AMF root colonization declined by 25%, 23%, 26%, 26%, and 11%, respectively, relative to a pea-wheat rotation (Nayyar et al., 2009). Furthermore, Bainard et al. (2017) revealed increases in general fungal pathogens (e.g., Fusarium avenaceum, F. redolens and Alternaria alternaria) and some crop-specific pathogens (e.g., Didymella pinodella and F. solani,) when two or more legumes were included in 4-year legume-cereal rotations. Overall, legume based rotations can bring many economic benefits to producers by increasing soil N content, enhancing soil C and P availability, modifying top soil water availability and breaking down cereal specific disease, which has been recognized worldwide especially in dry agricultural regions (Yigezu et al., 2019; Zhao et al., 2020). However, from the soil microbe point of view, types and frequency of legume crops applied in rotations should be carefully considered in order to optimize bio-functions of beneficial microorganisms in the soil.

#### 3.2. Canola based cropping systems and their impacts on soil microorganisms

#### 3.2.1. Canola based crop rotations can reduce some host specific pathogen circles

Canola (*Brassica napus* L. or *B. juncea* L.) is the third most important oilseed crop in the world after palm (*Arecaceae palmae*) and soybean, providing edible oil, fodder and biofuel production. Currently, annual global canola production is about 69 million tons, mainly from the European Union, Canada and China (Faostat, 2018). Canola-based rotations are common globally due to the high demand for vegetable oil and income for producers. When canola is followed by cereal crops in canola-based rotations, the yield of the following crops can increase due to a depression in the growth of some cereal-specific pathogens. For example, several rhizospheric and endophytic isolates from canola plants—classified as *Bacillus* spp.—exhibited antagonistic effects to wheat-related fungal diseases (Etesami and Alikhani, 2016). In a long-term study, several putatively plant growth promoting soil bacteria and fungi (e.g., *Serratia proteamaculans, Arthrobacter* spp., *Stenotrophomonas* spp., *Amycolatopsis* spp., *Pedobacter* spp., *Fusarium merismoides* and *Fusicolla* spp.) were favored by the canola phase in canola–legume–cereal rotations (Lay et al., 2018). In particular, *S. proteamaculans* is a well-known PGPR that promotes nodulation and nitrogen fixation in lentils and soybean, and *Pedobacter* spp. is a plant-growth-promoting endophyte in canola and some cereal crops that can produce and deliver indole acetic acid (IAA) to host plants. *Fusarium merismoides* and *Fusicolla* spp. belong to genera containing many plant pathogens, but their abundance is correlated positively with canola yield. Meanwhile, *Olpidium brassicae*, a known pathogen and member of the Brassicaceae, was omnipresent in canola roots, but its relative abundance declined at high seeding densities (Lay et al., 2018), indicating selective effects of canola on some host specific soil-borne pathogens.

#### 3.2.2. Mineral fertilization and non-AMF colonization related to canola can restrict soil microbial community development

Concerns with canola-based rotations include intensive mineral N fertilization and the development of resistant pathogens due to intensive pesticide application in canola cropping (Van De Wouw et al., 2017). While *Brassica* species can break the life cycle of wheat-specific soil-borne pathogens relative to cereals such as barley (*Hordeum vulgare* L.), they offer fewer environmental benefits due to the use of high levels of mineral fertilizers and artificial pesticides associated with growing *Brassica* species. However, limited systematic studies are available on the long-term effects of canola on soil microorganisms and soil health. As a non-mycorrhizal crop, canola receives limited benefits from AMF and other well-known functional soil microbes, such as free-living nitrogen fixers that are associated with AMF mycelium growth. Consequently, large amounts of chemicals

(e.g., mineral N fertilization) are applied in canola production systems (Gan et al., 2012b), which negatively impact soil microorganisms and their biological functions. This is particularly relevant for soil microorganisms involved in N cycling processes. For example, Yang et al. (2015) revealed changes in bacterial functional gene pools related to denitrification during the canola stage in rotational experiments in Western Canada and reported a modified community structure of denitrifiers related to canola growth. In particular, the relative abundance of *nirK* and *nirS* genes increased by almost 100% and 35%, respectively, while the *nosZ* gene only increased by 50% with the high fertilization strategy, which indicates an increased potential for the release of the greenhouse gas nitrous oxide in canola fields. Another study revealed that N application in canola fields can strongly modify the abundance of *nirS* dominated denitrifiers (Yang et al., 2017). As a consequence, large amounts of mineral N- and P-fertilizers and pesticides applied in canola-based rotations in Canada accounted for over 80% of the estimated equivalent greenhouse gas emissions in this region (Gan et al., 2012b), which could very likely be due to changes of some critical functional soil microbial groups that are closely related to denitrification. Selecting appropriate following crops and agronomic activities in canola-based rotations is critical for optimizing canola yield and optimizing the functionality of the soil microbial community.

# 3.3. Wheat based cropping systems and their impacts on soil microorganisms

# 3.3.1. Low nitrogen fertilizer use efficiency and continuous wheat planting in wheat based rotations can largely increase specific soil pathogen growth

Wheat is one of the most widely grown field crops in the world, and the history of wheat production can be traced back centuries (Lawes and Gilbert, 1894). Since the Green Revolution, wheat has been popular worldwide, largely due to the availability of synthetic fertilization and pesticides that help increase crop yields and farm profitability (Angus et al., 2015). On a global scale, more than 100 M tons of N fertilizer are applied every year, with about 50% applied to the three major cereal crops—maize (*Zea mays* L.), rice (*Oryza sativa* L.) and wheat. However, the fertilizer-N recovery efficiency in these crops on a global scale is only 56%, 36% and 48%, respectively (Ladha et al., 2016). In some developing countries, N fertilizer input can be as high as 500 kg ha<sup>-1</sup>, but N-use efficiency is less than 33% (Zörb et al., 2018). Higher yield after fertilization leads to more plant residues returning to wheat fields, which increases soil organic matter, total N and C (Cong et al., 2015; Song et al., 2017), but decreases soil pH, lowers bacterial diversity and modifies bacterial community structure in soil (Wang et al., 2018). Studies show that microbial functional genes related to nitrification/denitrification can be elevated by long-term N fertilization in wheat fields (Abalos et al., 2014; Qu et al., 2014). Surprisingly, about 48% of the total global N budget from these three cereals over the last 50 years was from N resources other than fertilizers, including animal manure, decomposed plant residues and BNF (Ladha et al., 2016), therefore, microbial activities related to plant residue decomposition and non-symbiotic BNF are key in wheat based cropping systems.

One major concern for wheat-based rotations is the accumulation of cereal specific soil pathogens. Certain bioactive chemicals produced by cereal crops can influence the rhizosphere microbial community. By producing these compounds, wheat crops can select beneficial soil microorganisms from Proteobacteria, Actinobacteria and Firmicutes, which are positively related to wheat yield (Yang et al., 2012a). However, continuous wheat cropping in long-term rotations increase host-specific pathogens in soil, such as Gaeumannomyces graminis var. tritici, a fungal disease known as 'Take-all', which causes yield losses in wheat (Kwak and Weller, 2013). Some wheat cultivars can release phytochemicals into the soil to reduce the damage from these diseases. For example, benzoxazinoids, a group of secondary metabolites from several cereal crops, including wheat, maize and rye, have been reported for their biological disease control capabilities (Hu et al., 2018). Another example is 2,4-dihydroxy-7methoxy-1,4-benzoxazin-3-one (DIMBOA), a compound produced by wheat roots that attracts Pseudomonas putida, a rhizobacterium that triggers the emission of stress-induced volatile compounds inhibiting pathogens in shoots (Neal et al., 2012). Also, 6-methoxy-2-benzoxazolinone (MBOA) produced from cereals inhibited Fusarium gene expression by blocking the trichothecene biosynthetic pathway (Karagianni et al., 2015). However, these benzoxazinoids could trigger tolerance of these pathogens and inhibit non-target soil microbes that are sensitive to benzoxazinoids, thus modifying the soil microbial community in wheat field over the long term (Saunders and Kohn, 2008, 2009). In general, although wheat crops have the capacity to inhibit some host specific pathogens to some extent, wheat-based rotations should be carefully designed and proper break crops should be included in these rotations to break down cereal specific pathogen cycles.

# 3.3.2. Increasing diversification in wheat-based rotations is the key to enhance soil health and release soil disease pressure caused by wheat growth

Diversifying wheat-based cropping rotations can enhance the build-up of beneficial soil microbes that promote plant growth through different plant root morphologies, root exudation and carbon allocation that can influence the population density and bioactivity of soil microorganisms. The host-plant selective effect may be cultivar-specific (Bazghaleh et al., 2015) due to differences in the phytochemical profile among cultivars (Ellouze et al., 2012). The 'host preference' can impact the growth of beneficial microbes, such as PGPR and AMF, suggesting the importance of the selection of appropriate cereal crops based on the soil health in sustainable agroecosystems.

#### 4. Tillage alters the structure and functionality of soil microbial communities

#### 4.1. Different tillage practices can influence microbial respiration and enzyme production

Tillage is an important farming practice for loosening soils, controlling weeds, restricting soil-borne phytopathogens, promoting litter decomposition through the incorporation of plant residues and enhancing crop production, but also increases soil erosion, nutrient loss, greenhouse gas emissions, and the break down of fungal hyphal networks (Hobbs et al., 2008; Zuber and Villamil, 2016). No-till and conservation tillage practices are regarded as sustainable alternatives—widely used in many countries/regions, including Australia, Canada, the U.S., South America, Europe and Asia-due to the reduced disturbance of soil and increased return of residue which can improve soil physical structure, water retention capacity and reduce soil erosion (Deubel et al., 2011). The effects of tillage can vary substantially in terms of the soil microbiome (Table 2). No-till generally increases soil microbial biomass and microbial enzymatic activity compared to tillage, but is dependent on the type of tillage management and soil physiochemical properties. In particular, tillage with intensive disturbance such as moldboard and double-moldboard significantly reduced soil microbial biomass carbon and nitrogen compared to no-till, while tillage practices with light disturbance such as chisel did not show a significant difference from no-till (Zuber et al., 2018; Zuber and Villamil, 2016). Higher microbial respiration rates under no-till compared with tillage practices was detected based on a metabolic quotient (qCO<sub>2</sub>) study (De Andrade Barbosa et al., 2019). However, another study found that this effect became weaker after 10 years (Zuber and Villamil, 2016) indicating the soil microbial community potentially has the capacity to re-balance following disturbance. Soil enzymes that are indicators of microbial activity, such as fluorescein diacetate and dehydrogenase, didn't show significant relationships with tillage practices, while other enzymes involved in nutrient cycling such as  $\beta$ -glucosidase, urease, phosphatase and arylsulfatase, were significantly inhibited in the top layer (0–10 cm) of soil under tillage practices (Balota et al., 2014; Mijangos et al., 2006). However, enzyme activities largely depend on other factors such as soil depth, precipitation and soil physiochemical properties along with tillage practices (Zuber and Villamil, 2016).

#### 4.2. Tillage effects on soil microorganisms were largely due to changes in soil microclimate and soil nutrient availability

Most of the tillage related effects on the soil microbial community are primarily due to changes in the microclimate. Conservation tillage and no-tillage retain a cool, moist microclimate on the soil surface while tillage increases the soil surface temperature, stimulating microbial growth for residue decomposition (Zuber and Villamil, 2016). A 31-year study showed that no-tillage increased gram-positive bacteria, *Actinomycetes*, and AMF by 5%, 6% and 17% respectively, compared with the tillage treatment and reduced saprophytic fungi by 14% (Mbuthia et al., 2015). In the same study, no-tillage increased C, N and P cycling-related microbial-induced enzymes, namely β-glucosidase, β-glucosaminidase and phosphodiesterase, by 32%, 26% and 42%, respectively, indicating that no-tillage promoted more active nutrient cycling by soil microorganisms in the study area. Furthermore, compared to conventional tillage, reduced tillage has been associated with optimized soil functional bio-regulations by reducing *amoA* gene numbers and increasing the abundance of *Alphaproteobacteria* and *Actinobacteria*, which are closely related to many soil bio-functions, including xenobiotic biodegradation and greenhouse gas emissions (Babin et al., 2019). Proper control of tillage practices in rotations can help to establish healthier and more functionally beneficial soil microbial communities that can improve soil health, reduce reliance on synthetic inputs, and promote crop yield.

# 5. Disease control agents and their side effects on soil microorganisms

#### 5.1. Synthetic disease control agents often show non-target impacts on soil microorganisms growth

Minimizing plant diseases while preserving beneficial soil organisms is one of the main goals of modern agriculture. However, many disease control chemicals often impact non-target microorganisms in the plant-soil-microbe complex, as some pesticides target non-specific binding sites (Shao and Zhang, 2017; Yang et al., 2012b). Changes in the members of a microbial community may trigger shifts in the structure of the whole community as microbial taxa and targeted pathogens may have identical or similar metabolisms or functional roles (Yang et al., 2011). For example, carboxylic acid fungicides inhibit protein synthesis and DNA replication in some fungal pathogens but also in prokaryotic cells with DNA topoisomerase II as the nontarget affecting site of this class of fungicides (Sioud et al., 2009). Another example is a class of pesticides including glucopyranosyl antibiotics, which are toxic to non-target bacteria and possibly to higher eukaryotic organisms as these chemicals can inhibit the synthesis of certain amino acids (Carr et al., 2005). Also, there are some indirect impacts of agrochemicals on nontarget soil microorganisms that are functionally dependent on host plants. For example, foliar fungicides applied to chickpea-i.e., chlorothalonil, pyraclostrobin and boscalid-significantly increased the abundance of *Fusarium* in seminal roots of durum wheat grown after chickpea in rotations (Esmaeili Taheri et al., 2015). Therefore, chemical application for disease control in rotations could modify the structure of soil microbial communities, which may further impact their functions in soil.

Understanding the non-target effects of pesticides on beneficial microorganisms is key to assessing the environmental hazards of agrochemicals (Yang et al., 2011). Assessing the long-term impacts of pesticide use on soil microbiomes is particularly important for environmental sustainability. However, current legislation for pesticide use in cropping lacks the requirement for soil microbial tests; this needs to be improved. Soil tests for fertilization recommendations may not show the significant effects of pesticides, but this could be due to only a few bacterial species being evaluated in soil tests (Jacobsen and Hjelmsø, 2014). Improved evaluation procedures are urgently needed to optimize the functional capacity of the soil microbiome and to maintain healthy soils for the long term.

#### 5.2. Biocontrol agents may show low toxicity, but their application should still be used with caution

The use of biocontrol agents to replace chemical pesticides may be a better option for reducing the non-target effects of agrochemicals. Biocontrol agents offer many advantages over synthetic chemicals such as fewer impacts on non-target microorganisms, high efficiency in controlling pesticide-resistant pathogens, and low risk of developing resistance (Wei et al., 2016). However, the application of biocontrol agents in the field may lead to some unexpected consequences, such as restricting the growth of AMF and other beneficial microbes and breaking the ecological balance of the soil system. Non-target effects of biocontrol agents are mainly due to competitive displacement, toxicity and pathogenicity on non-target organisms (Brimner and Boland, 2003). Many biocontrol agents are living organisms that compete with native non-target microbial species for ecological niches and soil resources to survive. For example, Pseudomonas fluorescens strain CHAO used as a biocontrol agent had a strong inhibiting potential on native rhizosphere bacteria isolated from barley due to competition for limited nutrients and space with indigenous microbial species (Johansen et al., 2002). Pseudomonas putida WCS358r reduced soil-borne fungal diseases by producing the antibiotics phenazine-1-carboxylic acid (PCA) and 2,4-diacetylphloroglucinol (DAPG). These antibiotics can inhibit many other saprophytic fungi and bacteria in soil (Bakker et al., 2002). Some microbial taxa, albeit designed as biocontrol agents, may show pathogenicity to the host plant under certain conditions. An in vitro antagonism test showed that several selected strains of Microdochium bolleyi inhibited F. graminearum, but once F. graminearum was controlled, these M. bolleyi strains had considerable pathogenicity to the host plant (Comby et al., 2017). Some biocontrol agents are reported for their allergenicity on humans, as some of the chemical compounds they produce, such as cyclosporine A and mycotoxins, may result in IgE-mediated allergies and down regulation of immune responses (Konstantinovas et al., 2017).

Most current research on biocontrol agents is based on short-term studies. Longer-term observations are needed to understand the mechanisms and consequences involved in pathogen control and virulence (St. Leger and Wang, 2010). Many studies have shown that genetically engineered biocontrol agents, such as mycopesticides and mycoacaricides, are advantageous due to their cost-effectiveness, easy-to-use formulation, and efficacy (Wang and Feng, 2014). However, the use of genetically modified strains in the field may raise safety concerns, not only for non-target impacts but unpredictable long-term ecological influences due to possible horizontal gene transfer from alien strains to native species, which frequently occurs in nature (Wrzosek et al., 2017).

#### 6. Fertilization shifts the functional capacity of soil microorganisms

Soil nutrient availability is one of the most important factors for crop growth. Mineral fertilizers have been used for crop production worldwide for decades, which has been estimated to reach 200 million tons annually by 2050 in order to increase crop yields to meet global demands (FAO, 2012). Long-term fertilization practices can significantly alter soil biological properties, soil eco-functions and soil quality (Bhatt et al., 2016, Zhao et al., 2016). Mineral fertilizers incorporating N, P and K macronutrients for crop productivity (Cordell and White, 2014; Das et al., 2019) interact with soil microbial communities (Table 3). In a Black-Box Ecosystem study, Allison and Martiny (2008) reported significant changes in soil microbial community composition from mineral fertilizer (N/P/K) inputs in 30 of 38 projects. More importantly, soil nutrient availability can shift the structure and function of soil microbial communities, including AMF (Bainard et al., 2014). How these effects interact with other human activities in long-term crop rotations remains unclear.

## 6.1. Mineral fertilization can increase crop productivity, but inhibit soil microorganism growth and restructure microbial communities

#### 6.1.1. Nitrogen fertilization

Mineral N fertilizer use may have exceeded the planetary boundary of N recycling and already caused extensive damage in many ecosystems (Stevens, 2019). Net primary production in natural terrestrial soil systems is usually N limited, and increasing N inputs can boost soil microbial growth (Wardle, 1992). However, N inputs in agroecosystems are usually applied at much higher rates than needed to achieve high crop yields, which has led to negative environmental impacts (Yu et al., 2019). Repeated N fertilization can lead to high soil osmotic potentials and NH<sub>3</sub> concentrations, and soil acidification, which directly affects microbial community diversity and composition (Geisseler and Scow, 2014). Microbial biomass generally declines with long-term N fertilization (Treseder, 2008), which can further impact the structure and function of the soil microbial community. It has been reported that high N fertilization can enhance the growth of root-derived C dependent soil microbes but limit the growth of soil-derived C dependent microbes by altering plant growth, which can restructure the soil microbial community (Bicharanloo et al., 2020). A long-term rotation experiment, increased N application rates decreased the abundance of denitrifiers belonging to  $\alpha$ -proteobacteria and increased those belonging to  $\gamma$ -proteobacteria, and changed the amount of nirS and nirK dominated denitrifiers (Yang et al., 2015). Bowman et al. (2018) found that N fertilization significantly changed plant species composition in alpine ecosystems and the concentration of soil NO<sub>3</sub><sup>-</sup>, which increased the nitrification rate and reduced the diversity of bacteria and fungi. In addition, the effects of N fertilization carried over after cessation of fertilization-9 years after fertilizer cessation, the nitrification rate in the 60 kg N ha<sup>-1</sup> treatment remained 108% higher than the 0 kg N ha<sup>-1</sup> treatment. These findings suggest that some soil microbial variables do not return to baseline levels after long-term cessation of N fertilization.

# 6.1.2. Phosphorus fertilization

Mineral P fertilizer is also a frequently used plant nutrient, which is vital to cell membranes, nuclear molecules, and fruit/ seed development (Cordell and White, 2014). Crop plants can reach soil available P through three pathways: plant storage pathway, biochemical rhizosphere modification pathway and soil microbial pathway (Hallama et al., 2019), which will be influenced by mineral P application. Zhong et al. (2010) found long-term P fertilization was positively related to total bacterial PLFAs and anaerobic bacterial PLFAs, and affected the phospholipid fatty acid content (PLFA) of soil microbial communities which may be related to soil P cycling. Phosphorus application can directly shift the structure and functionality of P cycling related soil microbes, as the deficiency in soil available P triggers the bacterial community to develop increased P solubilization capacity. For example, bacteria such as *Burkholderiales* and *Massilia* spp. can produce exopolyphosphatase and acid and alkaline phosphatases which triggers P solubilization and mineralization, respectively, are more dominant in P-deficient soils (Samaddar et al., 2019). Moreover, high P application rates can boost the growth of these microorganisms which can use more root-derived C resources, thus modifying total soil microbial C use efficiency, microbial biomass C pool, microbial turnover rate and eventually changing total soil organic C as a consequence (Bicharanloo et al., 2020). Overall, P fertilization can remarkably impact these functional microbial groups that play essential roles in soil P cycling, which can further influence other soil nutrient availabilities. Balancing the amount of mineral P fertilizer input and biological P solubilization appears to be the key for establishing sustainable P management strategies.

# 6.1.3. Potassium fertilization

Potassium is another important mineral nutrient for plant growth, as it has a close relationship with foliar stomatal functions, water conductance and the synthesis of many environmental stress-related proteins that can modify rhizosphere properties (Sardans et al., 2012; Zhang et al., 2016a). A 29-year fertilization experiment revealed that continuous K inputs decreased soil pH and available N while increasing available K in the soil (Zhang et al., 2018a, 2018b). More specifically, the diversity and size of the soil bacterial community increased, but the McIntosh dominance index (used to describe the heterogeneity of a sample in geometric terms) decreased, and the ability of these bacteria to use different carbon sources changed. In K-deficient soils, the application of K fertilizer boosted the abundance of functional genes involved in C, N and P cycling, and modified relative abundance of microbes involved in denitrification and ammonification in the soil (Zhang et al., 2018a, 2018b). However, the mineral fertilization effect on soil microorganisms varies with fertilizer type, application rate, environmental conditions and concurrent agronomic activities, which likely regulates the structure and functionality of soil microbial communities (Geisseler and Scow, 2014; Guo et al., 2019).

# 6.1.4. Soil carbon and organic matter changes due to mineral fertilization

Soil organic matter is a key component of soil physical and biological quality that favors and sustains biogeochemical cycling in the soil ecosystem (Woolf and Lehmann, 2019). Long-term mineral fertilization affects SOC in surface and subsurface soil layers. In particular, mineral fertilizer input increased SOC storage in the topsoil layer, but had no effect in soil deeper than 60 cm (Liang et al., 2019; Zhang et al., 2015). Considering that soil microorganisms are usually C limited, increasing SOC would boost growth of the soil microbial community in general. A meta-analysis based on 107 datasets revealed that long-term mineral fertilization increased SOC, microbial biomass carbon content and modified soil pH, which varied among different types of fertilization (Geisseler and Scow, 2014).

# 6.2. Organic fertilization and biofertilization

#### 6.2.1. Organic fertilization can selectively alter the growth of soil microorganisms

In order to provide food with better quality and fewer chemical pollutants, organic fertilizers are widely used in agriculture either on organic farms or mixed with mineral fertilizers on conventional farms. In general, organic fertilization derived from animal excreta or vegetable residues have more complex components and balanced nutrients compared with mineral fertilizers. Accordingly, long-term organic fertilization practices promote microbial biomass (Fließbach et al., 2007), microbial species richness (Hartmann et al., 2015; Schmid et al., 2018), microbial activity (Lori et al., 2017) and the abundance of plant beneficial microbes (Francioli et al., 2016). However, the effect of organic fertilization on the soil microbiota is not consistent across previous studies and depends on the pedoclimatic context and the overall agricultural strategy applied at the site (Paul Chowdhury et al., 2019). The effects of long-term organic fertilization are reflected in specific soil microbial community composition or soil nutrient availability, and are therefore expected to affect belowground plant–microbe interactions and potentially aboveground plant performance (Van Der Putten et al., 2016). For example, Firmicutes was enriched and Alpha-proteobacteria was inhibited by long-term organic fertilization, resulting in a significantly different soil microbial community compared with long-term mineral fertilization in the lettuce rhizosphere (Paul Chowdhury et al., 2019).

### 6.2.2. Biofertilization can improve soil health, but should be applied with some caution

Another fertilization strategy is biofertilization, which contains plant-growth-promoting microbes (PGPM), has been used in sustainable agriculture to increase the yield and quality of agricultural products. The taxa included in biofertilizers are usually based on traits that improve plant growth and yield. In a Canadian study, a high concentration of PGPM inoculants effectively

improved crop growth and the end product (Lupwayi et al., 2000), but it was unknown whether this lead to structural changes in the soil microbial community. Although the potential influences of PGPM on the soil microbial community are usually minimal due to strong competition from the native microbial community, they would still actively compete with alien inoculants for soil nutrients and habitats on the root surface and in the rhizosphere. These interactions would either rebuild relationships or trigger succession of the native soil microbial community (Ambrosini et al., 2016). As host plants, crops provide a large amount of root exudates and hormones to the rhizosphere, which help to shape the rhizosphere microbial community. Since PGPM inoculants are usually designed to target crop hosts, they could be more competitive than native species under certain conditions and alter the native soil microbial community as a consequence. For example, inoculating two selected PGPM strains *Sinorhizobium meliloti* 4H41 and *Rhizobium gallicum* 8a3 in a common bean (*Phaseolus vulgaris* L.) field unexpectedly promoted the growth of some native *Rhizobiaceae*, *Firmicutes* and *Actinobacteria* species (Trabelsi et al., 2011). Although most of these changes occur in the short term, the use of these biofertilizers, especially genetically modified ones, should be handled with caution. For example, the activity of a genetically modified strain of *S. meliloti* Rm42 was detected 6 years after its first application in an alfalfa field, and its genetically modified plasmid was found in other local *S. meliloti* strains, presumably due to horizontal gene transfer and microevolution (Morrissey et al., 2002).

# 7. Consequences of anthropogenic interventions on the microbe-related carbon footprint

The carbon footprint of agroecosystems has increased dramatically in the last few decades as crop inputs have increased. The carbon footprint of crop production per unit farmland is the balance between the equivalent carbon emissions and carbon sequestration. Most anthropogenic interventions in agroecosystems, such as fertilization, pesticide applications and farming implement operations, contribute to the carbon footprint of crop production (Fig. 3).

Among anthropogenic activities, mineral fertilization is the most important contributor to the carbon footprint of cropping systems. In a 25-year long-term study in Saskatchewan Canada, Gan et al. (2014) reported that N fertilization accounted for 50% of the total CO<sub>2</sub> equivalent greenhouse gas emissions in wheat production, of which 22% comes from N<sub>2</sub>O emissions. In Australia, over 16% of nation-wide greenhouse gas emissions are a result of agricultural activities (Young et al., 2009). Transformation of ammonia to nitrate, and N<sub>2</sub>O and NO gases mainly rely on the activities of several soil microbes involved in nitrification and denitrification, such as the bacterial genera *Nitrosomonas, Nitrobacter, Pseudomonas* and *Clostridium*, and Archaea phylum *Thaumarchaeota*. Any impact of these soil microorganisms from anthropogenic activities could affect N<sub>2</sub>O emissions and change the carbon footprint (Table 4). Large inputs of mineral N fertilization in agroecosystems could shift the C/N balance in soil, leading to increased denitrification. However, due to C-resource limitations in N-rich soil, *nir-* and *nor-*gene clusters showed larger relative abundance than *nos-*gene clusters, which could preferentially increase N<sub>2</sub>O and NO emissions over N<sub>2</sub> emissions in the soil (Yang et al., 2015), aggravating the negative effect of N fertilization to some extent.

Furthermore, soil microorganisms also contribute to the transformation of CH<sub>4</sub>, the second most important greenhouse gas after CO<sub>2</sub>. The soil is a large sink for CH<sub>4</sub>, and CH<sub>4</sub> consumption is the result of cooperation among many soil bacteria, including methanotrophic archaea, ammonium-oxidizing bacteria and sulfate-reducing bacteria. A previous study reported that fertilization, especially bio-fertilizer applications, can minimize CH<sub>4</sub> emissions from soil, and long-term N fertilization could inhibit CH<sub>4</sub> oxidation as well, which may be mainly due to changes in the methanotroph community (Singh and Gupta, 2016). However, long-term fertilization effects on CH<sub>4</sub> emission and other environmental impacts still remain largely unknown.

An optimized crop diversification strategy in rotations could reduce the carbon footprint of agroecosystems because diversified systems generally use fewer fertilizer and pesticide inputs, require fewer interventions with farm machinery, and have better nutrient use efficiency and soil microbial function (Gan et al., 2011). For example, a meta-analysis based study revealed that conservation agriculture with high crop diversity and low soil disturbance agronomic practices can contribute to annual carbon sequestration over 500 Tg  $CO_2$  per year in Africa, which is about 95 times the carbon sequestration from current cropping systems (Gonzalez-Sanchez et al., 2019). In China, optimized fertilization strategies in cereal-based rotations can reduce N<sub>2</sub>O emission and total global warming potential over 16% and 9%, respectively (Lan et al., 2020). In cereal-based rotations, the extensive root system of cereal crops has a positive effect on soil microbial communities as it is a large source of C for soil systems sustaining all levels of the trophic chain (Zhang et al., 2016b), as carbon flow in cereal crop fields passes through higher trophic-level organisms, such as nematodes, earthworms and insects, rather than being more directly released by microbial respiration or leaching, as seen with plant residues. Crop sequences can also affect the carbon footprint. For example, Gan et al. (2012c) reported a high carbon footprint in spring barley following an oilseed crop due to high N fertilization and low N use efficiency in the previous crop in Western Canada. In a long-term rotation experiment, Gan et al. (2011a) showed that the carbon footprint of canola and wheat in rotation could be reduced by 13% with increased activity of AMF and P-solubilizing microbes. The same study also showed that cereal-oilseed rotations had a higher carbon footprint than cereal-legume rotations, partially due to the lower contribution of diazotrophs to the system. On the other hand, the carbon footprint of pesticide application is usually higher in broadleaf crop based rotations than in cereal crops, as severe disease pressure often occurs in oilseed and legume crops (Liu et al., 2016), which could lead to side effects for soil microorganisms. There are many other anthropogenic interventions that can influence the carbon footprint of crop rotation systems, including crop intensification (Gan et al., 2012a), intercropping strategies (Hauggaard-Nielsen et al., 2016), cropping tactics (Peter et al., 2017), and the synchrony between nutrient inputs and crop demand (Alhajj Ali et al., 2017).









#### 8. Conclusion

This review showed that anthropogenic activities have a remarkable influence on microbial communities in agroecosystems, which affects environmental sustainability, economic activities and human society (Fig. 4). The contribution of soil microbes to agroecosystem services and crop productivity rely on functional diversity, abundance and colonization rate of these microorganisms (Ryan and Graham, 2018), and their biological and ecological functions are critical in environments which cannot be ignored, considering the important roles they play in soil systems. We showed that soil microbial communities are more influenced by many long-term agricultural practices than often assumed, but limited long-term field studies are available, and our ability to manage beneficial microorganisms to favor crop yield is compromised of a rudimentary knowledge of many important aspects of their growing habits and symbiosis, and how they function in complex ecosystems. These include the determinants of colonization of beneficial microorganisms for crop genotypes; the impact of soil available nutrient resources balance on microorganisms in the field; and the complexity of interactions among crop diversity, agronomic activities, and the composition of soil microbial communities. This review summarized valuable knowledge in understanding the influence of crop rotations and soil microbial communities and their potential functional activities, especially long-term, highlighting the problems of improper use of break crops. This will hopefully draw attention from producers and policymakers to the importance of maintaining soil health in agroecosystems. We suggest that adjusting anthropogenic activities in agriculture to favor the abundance and functional diversity of soil microorganisms should be considered a top priority. Optimizing agricultural productivity in a sustainable manner should be achieved through rectifying soil nutrient deficiencies, increasing crop diversification in rotations or in space, efficient use of synthetic chemical and biofertilizer applications, and agronomic practices. Ideally, long-term field experiments should be conducted in collaboration with local producers and researchers to yield sufficient knowledge for further policymaking.

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# **Conflict of Interest**

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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