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Minireview

Agroecological transition: towards a better understanding of the impact of ecology-based farming practices on soil microbial ecotoxicology

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Abstract

Alternative farming systems have developed since the beginning of industrial agriculture. Organic, biodynamic, conservation farming, agroecology and permaculture, all share a grounding in ecological concepts and a belief that farmers should work with nature rather than damage it. As ecology-based agricultures rely greatly on soil organisms to perform the functions necessary for agricultural production, it is thus important to evaluate the performance of these systems through the lens of soil organisms, especially soil microbes. They provide numerous services to plants, including growth promotion, nutrient supply, tolerance to environmental stresses and protection against pathogens. An overwhelming majority of studies confirm that ecology-based agricultures are beneficial for soil microorganisms. However, three practices were identified as posing potential ecotoxicological risks: the recycling of organic waste products, plastic mulching, and pest and disease management with biopesticides. The first two because they can be a source of contaminants; the third because of potential impacts on non-target microorganisms. Consequently, developing strategies to allow a safe recycling of the increasingly growing organic matter stocks produced in cities and factories, and the assessment of the ecotoxicological impact of biopesticides on non-target soil microorganisms, represent two challenges that ecology-based agricultural systems will have to face in the future.

Keywords: biopesticides; microbial ecotoxicology; microplastic; organic fertilizers; sustainable agriculture

Introduction: agroecology in the frame of microbial ecotoxicology

Agroecology is as old as agriculture (Altieri 1987) and can be defined as the application of ecological concepts and principles (the study of interactions between plants, animals, humans and the environment) in the design and management of sustainable food systems (Gliessman 2007). The resulting set of agricultural practices seeks ways to improve agricultural systems by harnessing natural processes, creating beneficial biological interactions and synergies amongst the components of agroecosystems, minimizing synthetic and toxic external inputs and using ecological processes and ecosystem services (Wezel et al. 2020). There are currently many movements of ecology-based agriculture, sharing a grounding in ecological concepts: agroecology, biodynamic farming, organic agriculture, regenerative agriculture, conservation agriculture, permaculture. As developed by Gliessman et al. (2022), agroecology is not one of the alternatives, but rather an umbrella under which alternative systems can find support and commonality and participate in the movement to transform food systems.

Soil microorganisms are both the most promising and the most unknown components of the agroecosystem. Of the estimated total number of species, less than 1.5% of bacteria, and between 1.9 and 6.5% of fungi have been described (Orgiazzi et al. 2015). But they are increasingly considered as “little farmhands” (De Vrieze 2015), providing numerous services to plants and soils, including growth promotion, organic matter decomposition, nutrient supply, tolerance to environmental stresses and protection against pathogens and pests (Lemanceau et al. 2015, Trivedi et al. 2020). Given the utmost importance of microorganisms in agricultural systems, knowing and “driving” these communities for an optimization of ecosystem processes represents a major scientific front for the development of agroecology. It also appears essential to evaluate the impact of agricultural practices on them, and especially with regards to their exposure to synthetic or bio-based pollutants. Microbial ecotoxicology is a field of research that studies both the ecological impacts of pollutants on the various functions microorganisms ensure in their environment and the role of these microbes in the transfer and the degradation of the pollutants (Ghiglione et al. 2016). To our knowledge, no review has been made so far on the ecotoxicological impact of the different ecology-based agricultural practices on soil microbes. In order to do so, we divided the ecology-based systems into a set of cultural operations according to Wezel’s (Wezel et al. 2014)

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classification of main categories of practices (Table 1). For each category, we described the applicable official norms or principles attached to the practice in the 5 ecology-based systems: the DABFS standard for biodynamic farming; the IFOAM norms for organic farming; the papers of Wezel et al. (2014), HLPE report (2019), Wezel et al. (2020) and the website agroeco.org for agroecology; the book of Holmgren (2002), the paper of Krebs and Bach (2018) and website permaculture.org for permaculture; from the FAO website www.fao.org/ag/ca/1a.html for conservation agriculture. Unlike other alternatives, regenerative agriculture has no centralizing authority, widely recognized set of defining principles or promotional organization (Gliessman et al. 2022). We consequently didn't include this movement in the present review. Finally, we identified the substances presenting potential ecotoxicological risks, in the different systems and practices.

Among the six categories of practice, three were identified to present potential direct ecotoxicological risks: (i) crop fertilization; (ii) tillage and mulching; and (iii) crop protection. We identified the three other categories as having a potential indirect ecotoxicological effect, through their impact on the other three categories: (iv) practices addressing crop choice, crop spatial distribution, and crop temporal successions; (v) irrigation practices; and (vi) landscape element management. Indeed, these practices act on a reduction of weed, pest and diseases and thus, lead to a reduced use of pesticides and fertilizers. In addition, we added a category of practice: livestock management, as it impacts the quality of organic fertilizers. In this review, we aim to evaluate the ecotoxicological consequences of the first three categories of ecology-based farming practices on soil microorganisms. We also identify the pitfalls and research gaps.

Benefits and risks of organic fertilization

Since the origins of agriculture, application of organic matter was used to compensate for nutrient exports and restore soil fertility. This practice has been progressively set aside since the 1950 s in favor of synthetic fertilizers, efficiently increasing crop yield. However, after 70 years of practice, global assessments reveal that inorganic fertilizers significantly contribute to greenhouse gas emissions due to their production, transport and use, their leaching potential and, therefore, their implication in eutrophication and health hazards (Tilman et al. 2002). They can be a source of pollution, notably P fertilizers which often contain significant amounts of cadmium, mercury, and lead (Bünemann et al. 2006). There is a substantial body of literature indicating that long-term use of mineral fertilizers threatens soil fertility, mainly through soil organic matter (SOM) content decrease (Lal 2015, Huang et al. 2019). Building SOM is one of the main goals of soil management in ecology-based farming practices. SOM is paramount to sustain soil physical, chemical and biological fertility, and hence sustainable agricultural production. It feeds the soil food web, increases cation exchange capacity, improves soil texture and water retention capacity (Baldock and Broos 2012). SOM is considered as the main factor governing levels of microbial biomass in soil, followed by soil pH (Wardle 1992). Sources of new organic matter must continually be added to agricultural land to restore and/or maintain SOM stock, as farming practices tend to deplete it through harvest and decomposition.

There is a vast variety of organic fertilizers, that can be divided into five major categories (Goss et al. 2013): (i) livestock manure; (ii) municipal biosolids and septage (subject to regulatory control); (iii) green manure and crop residues; (iv) food residues and waste; (v) waste from manufacturing processes (e.g. residual organic material from pressing oil seeds, fish offal, dried blood, paper-mill biosolids, sugar beet sugar extraction). Organic fertilizers are also sometimes referred to as Organic Waste Products (OWP): complex mixtures composed of several constituents (some of them unknown) from different sources that can contain hazardous substances affecting soil functioning (Renaud et al. 2017). The ecology-based systems differ in their regulations of fertilization (Table 1). The less stringent system is conservation agriculture, having no regulation on fertilization. The most stringent system is Biodynamic farming, prohibiting synthetic fertilizers or fertilizers made soluble by chemical methods, as well as any materials that may contain contaminants or toxins. Organic agriculture is intermediate, and prohibits sewage sludge and synthetic fertilizers. The use of materials containing contaminants and toxins, as well as synthetic fertilizers are not in adequacy with the principles of agroecology and permaculture. In the strictest systems, organic fertilization therefore does not present ecotoxicological risks. However, OWP recycling is a big challenge for agroecology and the circular economy model. Many modern OM sources contain contaminants that may represent an ecotoxicological risk for soil microbes, particularly when used on the long term (as some contaminants accumulate in the soil). As reviewed by Bünemann et al. (2006), Goss et al. (2013), and Urra et al. (2019), the concentration in contaminants of OWP depends on their nature, origin and treatment (Table 2): animal-derived OWP, such as sewage sludges (biosolids) and livestock manure often contain active residues of therapeutic agents used to treat or cure diseases in humans and animals. They also often contain heavy metals such as copper (Cu), zinc (Zn), or cadmium (Cd), especially when industries contribute to the waste stream or when livestock feed is supplemented in Cu and Zn. Manures and sewage sludge generally have a higher salinity than municipal garden wastes and salts can build up in soil with repeated applications. Animal-derived OWP can also be a source of biological contaminants: pathogenic bacteria, viruses and parasites, as well as antibiotic resistant genes (ARG) and bacteria (ARB). Plant-derived OWP, such as green wastes from farms and gardens, have typically lower nutrient concentrations than manures or sewage sludges and may contain residues of synthetic compounds such as herbicides, insecticides, fungicides, and plant growth regulators. In addition, other trace organic pollutants might also be found in OWP such as personal care products (parabens, formaldehyde, PFAS, triclosan, diethyl-metatoluamide), industrial chemicals (polychlorinated biphenyls, phthalates, solvents) and unintentional by-products of industrial processes (dioxins and furans). These different categories of OWP can be used as a mixture, like in municipal solid wastes (MSW) and undergo two types of treatments to improve their properties, i.e. composting and anaerobic digestion. Composting, is generally accepted as a rapid and simple process to stabilize and reduce the waste mass, and anaerobic digestion as being energy efficient (Odlare et al. 2011). Both processes were also reported to effectively reduce human pathogens load within the digested organic material, total or partial degradation of antibiotic residues, and to degrade some but not all persistent organic pollutants (POP) (Bünemann et al. 2006, Hargreaves et al. 2008, Urra et al. 2019). The relatively high temperatures reached during composting processes may also decrease the load of ARB and ARGs, unlike anaerobic digestion (Urra et al. 2019). As metals are non-degradable, the best method of reducing their concentration and improving the quality of composts and digestates is early source separation (Hargreaves et al. 2008, Kupper et al. 2014). However, there is good experimental evidence demonstrating the decrease of metal bioavailability with the period of composting and maturation time (although the

Table 1. Guidelines per main category of practice, for the five ecology-based agriculture systems evaluated in this review. Acronyms: CA—conservation agriculture; SS—sewage sludge; MT—minimum tillage; GMO—genetically modified organism; Pr.—principles.

Categories of practices	Biodynamic farming[1]	Organic agriculture[2]	Agroecology[3]	Permaculture[4]	Conservation agriculture[5]
1. Fertilization	**Requirements** **Use only substances that are on an allowed products list**: appendix B of the DABFS. • **Plant-derived fertilizers** • **Animal-derived fertilizers**: manure (max 56 manure unit/acre), fish, bone meal, processing by-products • **Microbiological-derived fertilizers** • **Legumes/nutrient catch crops** • **Biodynamic preparations** refer to appendix J of the DABFS • **Biodynamic compost** • **Naturally occurring mineral fertilizers** (Rock dust, clays, lime fertilizer) **Highly regulated** • **Amount** of fertility that can be imported and applied • **Origin** of the fertilizer: distance from the farm, off-farm manure sources should come from certified organic livestock production minimum • Raw manure/urine • Approved P and K **salts**, Mg **sulphate**, **sulfur and trace minerals** **Prohibited** All products not on the list • **Synthetic fertilizers** or fertilizers made soluble by chemical methods, e.g. urea, superphosphates, sodium (chilean) nitrate • **Any materials that may contain contaminants or toxins**: organic wastes from municipal and industrial sources (SS), or from synthetic, chemically farmed agriculture	**Requirements** **Use only substances that are on an allowed products list**: appendix 2 of the IFOAM. • **Plant-derived fertilizers** • **Animal-derived fertilizers**: manure, blood, bone meal, fish product, etc. • **Microbiological-derived fertilizers** • **Compost and worm compost** • **Nitrogen fixation from plants** • **Biodynamic preparations** **Highly regulated** • **Naturally occurring mineral fertilizers** (Rock phosphate, Elemental sulfur, Potassium sulfate). Only as a supplement to biologically-based fertility methods, use restricted to cases where nutrient deficiency is documented by testing or diagnosed by an independent expert **Prohibited** All products not on the list • **Sewage sludge** • **Synthetic fertilizers** or fertilizers made soluble by chemical methods, e.g. urea, superphosphates, sodium (chilean) nitrate	**Applicable principles** **Pr. 1. Recycling** **Pr. 2. Input reduction** **Pr. 3. Soil health** Fertilization practices are diverse and adapted to local conditions and needs, but **prioritize the use of natural and organic sources of nutrients.** • **Plant-derived fertilizers** • **Animal-derived fertilizers** • **Microbiological-derived fertilizers** • **Compost and worm compost** • **Split fertilization** (to reduce the amount used) • **Mineral fertilizers** **Discouraged** **Does not comply with the principles:** • **Synthetic fertilizers** • Any materials that may **contain contaminants or toxins**, including sewage sludge	**Applicable principles** **Pr. 2. Catch and Store Energy**—Organic mulch application. **Pr. 5. Use and Value Renewable Resources and Services**—Legumes and animal manure as nutrient source, Mycorrhizal fungi. **Pr. 6. Produce no Waste**—Animal manure, Human excreta, Waste products as animal feed.	**Applicable principles** **Pr. 2. Keeping the soil covered—**crop residues are left on the soil surface. **No fertilizer limitation** fertilizers aren't part of the three CA fundamental principles.

Table 1. Continued

Categories of practices	Biodynamic farming[1]	Organic agriculture[2]	Agroecology[3]	Permaculture[4]	Conservation agriculture[5]
2. Tillage management	• Light tillage is allowed **Prohibited** • Bare tillage year-round	• No tillage limitation **Recommendations** • Prevent erosion and minimize loss of topsoil (MT, maintenance of soil plant cover, etc.)	**Applicable principles** **Pr. 2. Input reduction (petrol)** **Pr. 3. Soil health** • Reduced or no tillage • Direct seeding into cover crops/mulch	**Applicable principles** No applicable principle	**Applicable principles** **Pr. 1. Minimum mechanical soil disturbance** (reducing or eliminating tillage).
3. Weed, pest and disease management	**Requirements** **Use only substances that are on an allowed products list:** appendix C of the DABFS. • **Biological pest control:** Natural enemies, Trap, pheromones, repellents • **Botanical pesticides**: plant preparations, plant oils • **Microbial pesticides** (such as Bacillus thuringiensis (Bt) and granuose virus) • **Animal-based pesticides:** milk products, propolis, etc • **Other**s: homeopathic preparations, soft soap **Highly regulated** • **Mineral-based pesticides:** derived from minerals, such as copper salts (Max 3 kg Cu/ha per year), silicates, sulfur, potassium bicarbonate, Fe(III) Orthophosphate **Recommendations** Avoid biocides that are not selective to the pest species **Prohibited** Substances that do not appear on appendix C of the DABFS • **Synthetic pesticides** • **Growth hormones**	**Requirements** **Use only substances that are on an allowed products list**: appendix 3 of the IFOAM. • **Biological pest control:** Natural enemies, Traps, barriers, repellents • **Botanical pesticides**: Plant preparation, plant oils • **Microbial pesticides** (such as Bacillus thuringiensis (Bt) and Spinosad) • **Animal-based pesticides:** animal oils, beeswax, etc • **Others**: Biodynamic preparations, soft soaps, etc. **Highly regulated** • **Mineral-based pesticides:** derived from minerals, such as copper salts (Max 6 kg Cu/ha per year), silicates, sulfur, etc. **Prohibited** Substances that do not appear on Appendix 3–IFOAM norms. • **Micronutrients** in either chloride or nitrate forms • **Synthetic pesticides** • **co-formulants** that are carcinogens, mutagens, teratogens or neurotoxins	**Applicable principles** **Pr. 1. Recycling** **Pr. 2. Input reduction** **Pr. 3. Soil health** **Pr. 5. Biodiversity** **Pr. 6. Synergy**Crop protection seeks for an optimization of interrelated positive processes and mechanisms (based on natural enemies and biodiversity) within the farm, to limit the risks of infection or high prevalence of bio-aggressors, while minimizing synthetic and toxic external inputs. • **Botanical and microbial pesticides** • **Biological pest control** • **Allelopathic plants** in crop rotation • **Other biopesticides** **Discouraged** **Does not comply with the principles)** • synthetic pesticides	**Applicable principles** **Pr. 4. Apply Self-Regulation and Accept Feedback**—Enhancement of regulating ecosystem services.	**No specific pesticide limitation** Pesticides are not part of the three CA fundamental principles. However, integrated pest management (IPM) is often recommended. Herbicides are the most commonly used pesticides in CA, due to the weeds infestations problems.

concentration increases with digestion and composting) (Smith 2009).

We found sixteen global meta-analyses of the impact of OWP on soil microbes. Despite a very high residual heterogeneity, they reveal that organic fertilization has overall a positive effect on microbial communities compared to mineral fertilization. OWP application led, on average, to a 32%–51% increase in soil microbial biomass carbon, 24%–55% increase in microbial biomass nitrogen and 59%–95% increase in total phospholipid fatty-acids compared to conventional systems (Kallenbach and Grandy 2011, Geisseler et al. 2017, Lori et al. 2017, Zhang et al. 2017, Luo et al. 2018, Ren et al. 2019, Wang et al. 2021, Morugán-Coronado et al. 2022). Organic fertilization also had a positive impact on soil microbial diversity and community structure compared to mineral-only fertilization, with an average 3.0%, 10.2%, and 6.7%, increase in microbial Shannon, richness, and phylogenetic diversity, respectively (Shu et al. 2022), between 2.4% and 5% increase of the alpha diversity of soil bacteria, but no significant or negative effect on fungal alpha diversity (Bebber and Richards 2022, Shu et al. 2022). Microbial community activity is also positively impacted by organic

Table 1. Continued

Categories of practices	Biodynamic farming[1]	Organic agriculture[2]	Agroecology[3]	Permaculture[4]	Conservation agriculture[5]
4. Crop irrigation	**Recommendations** Irrigation needs are required to be met based on a strategy that emphasize water conservation. • Alternative pumping methods (solar, wind,...) • Irrigation scheduling • Irrigation water should be free of chemical contamination	**Recommendations** Organic management ensures that water resources are used sustainably.	**Applicableprinciples** **Pr. 1. Recycling** **Pr. 2. Input reduction** Adapt the agrosystem to the local water constraints. Use technologies like drip irrigation to increase water use efficiency.	**Applicableprinciples** **Pr. 2. Catch and Store Energy**—Rainwater harvesting measures **Pr. 5. Use and Value Renewable Resources and Services**	**No specificcrop irrigation limitations** Irrigation is not part of the three CA fundamental principles.
5. Crop choice, spatial distribution and temporal succession	**Requirements** • Use of **seeds coming from Biodynamic sources** if possible • **Crop rotation** • **Intercropping** • Use of crop residues and/or a cover crop for **permanent ground cover** • **Botanical species diversity** • **Predator habitat** • **Timing** of planting according to pest life cycle **Prohibited** • **Monoculture** • Planting the same crop for **more than 2 years in a row** • **Hybrid varieties** • **GMO and treated seeds** • **Nanotechnology**	**Recommendations** • **Crop rotation** • **Intercropping** • **Companion planting** (control pests and diseases naturally) • **Use organic seed and planting materials** (unless unavailable) **Prohibited** • **GMOs** • **Irradiation** • **Synthetic growth regulators**	**Applicable principle** **Pr. 5. Biodiversity** **Pr. 6. Synergy** The objective is to create beneficial biological interactions and synergies amongst the components of agroecosystems. • **Diversity of crops** • **Crop rotation** (including cover crops and leguminous plants) • **Intercropping and relay intercropping** • **Agroforestry** with timber, fruit or nut trees	**Applicable principle** **Pr. 4. Apply Self-Regulation and Accept Feedback**—Enhancement of regulating ecosystem services **Pr. 8. Integrate Rather than Segregate**—Polyculture (crops) **Pr. 10. Use and Value Diversity**—Plant species, Pollinator, Habitat, ... **Pr. 11. Use Edges and Value the Marginal**—High field border density, Field margins, Edges with forests	**Applicable principle** **Pr. 2. Keeping the soil covered.** Use of cover crop for permanent ground cover. **Pr. 3. Species diversification and crop rotation.** Lengthening and diversifying crop rotations, often by including legume crops. The crop sequences and associations must involve at least three different crops.
6. Management of landscape elements	**Requirements** • Minimum of 10% of the total effective land set aside as a **biodiversity reserve** • **Buffer zones** must be created between certified fields and chemically treated acres **Forbidden** • **Clearance of virgin forest**	**Recommendations** • **Maintain or enhance biodiversity** in crop and non-crop habitats on the farm holding. • **Protection of natural enemies of pests** through provision of favorable habitat, such as hedges, nesting sites and ecological buffer zones.	**Applicable principle** **Pr. 5. Biodiversity** **Pr. 6. Synergy** Integration of semi-natural landscape elements at field, farm and landscape scale (planting and management of vegetation strips and hedges in fields and at field borders).	**Applicable principle** **Pr. 2. Catch and Store Energy**—Woody elements in agriculture **Pr. 4. Apply Self-Regulation and Accept Feedback**—Enhancement of regulating ecosystem services **Pr. 9. Use Small and Slow Solutions**—Agroforestry systems **Pr. 10. Use and Value Diversity** **Pr. 11. Use Edges and Value the Marginal**—High field border density, edges with forests	**Landscape management not taken into account**

Table 1. Continued

Categories of practices	Biodynamic farming[1]	Organic agriculture[2]	Agroecology[3]	Permaculture[4]	Conservation agriculture[5]
7. Livestock management	**Requirements** • **Integrating livestock** into agronomic systems (except for approved exemption) • Have a **mixed livestock** population to sustain a self-sufficient system • Access to **free range forage and shelter all year** (refer to appendix F of DABFS for max stocking rates) • Min of 50% of the feed ration must come from on-farm production, the rest must be certified biodynamic or organic **Recommendation** • Herbal, homeopathic and anthroposophical treatment **Prohibited** • Genetically engineered animals • Hormonal treatments • Supplementation with synthetic amino acids • Antibiotics treatment and in feed • Routine and preventive treatments with allopathic medication (except vaccination required by law) • Totally slatted floors • Mutilation	**Requirements** • Animal production systems raise animals **organically** from birth or hatching • Animals are allowed **to graze in open pastures**, and their living conditions are kept **clean and comfortable** **Prohibited** • Prophylactic use of antibiotics and other allopathic chemical veterinary drugs • Growth hormones • Synthetic feed rations (amino acids, nitrogen compounds, stimulants, appetizers, preservatives, colouring agents, or any solvent-extracted substance) • Mutilation	**Applicable principle** **Pr. 1. Recycling** **Pr. 3. Soil health** **Pr. 4. Animal health and welfare** Integrating livestock back into agronomic systems **Discouraged** **Does not comply with the principles** • Confined animals • Antibiotics and other drugs treatment and in feed • Mutilation • Etc.	**Applicable principle** **Pr. 8. Integrate Rather than Segregate**—Integration of livestock, fish, and other animals	**Livestock management not taken into account**(system only for crops)

[1] From the Demeter association inc. biodynamic farm standard, 2017 (DABFS). Biodynamic farming is certified. Website: *biodynamics.com*

[2] From IFOAM-Organics International—*IFOAM norms for organic productions and processing*, version 2014, Germany. Organic farming is certified. Website: *Ifoam.bio*

[3] From the paper of Wezel et al 2014, Wezel et al 2020, and the HLPE report (2019). There is no agroecological certification. Website: *agroeco.org*

[4] From Holmgren (2002) and Krebs and Bach 2018. Instead of a farm certification, the certification is ensured through a Permaculture Design Certification Courses. Website: *permaculture.org*

[5] From the FAO website: *http://www.fao.org/ag/ca/1a.html* There is no CA certification.

fertilization, with increases in the activity of enzymes involved in soil hydrolytic C acquisition (39%), N acquisition (22%), P acquisition (48%) and oxidative decomposition (58%) (Luo et al. 2018), or more specifically dehydrogenase (74%), urease (32%) and protease activity (84%) (Lori et al. 2017). Application of organic fertilizer resulted in 46% more arbuscular mycorrhizal fungi (AMF) biomass relative to synthetic-only fertilization and was less detrimental to AMF richness than mineral-only fertilization (Jiang et al. 2021). Soil microbial functional diversity was 3.8% greater under organic than mineral fertilization (Bebber and Richards 2022). These global positive answers might however mask a negative effect of contaminants contained within the OWP. Of the above-mentioned meta-analysis, most focus on manure and plant-derived materials and their composts. Of the six taking into account urban and industrial wastes and sewage sludge, five present the individual effect of solid wastes and sewage or their digestate (Charlton et al. 2016, Luo et al. 2018, Jiang et al. 2021, Karimi et al. 2022, Shu et al. 2022). These studies reveal a very variable effect of urban and industrial organic wastes and sewage sludge on the microbial parameters. Negative effects on microbial parameters were also detected, especially for sewage sludge and digestates, so that we can not conclude the absence of any ecological risk of these products on soils (Charlton et al. 2016, Karimi et al. 2022). We can hypothesize that the great discrepancy between studies might be due to the differences in contaminant contents. However, none of these meta-analysis took into account the content of contaminants in the different fertilizers tested, except for one, testing specifically the impact of contaminated *vs.* uncontaminated sludge on soil microbial biomass (Charlton et al. 2016). The latter revealed that for soils

Table 2. List of the contaminants/substances identified in this review, the agroecological practice involved, the behavior in the soil and ecotoxicological impact of the contaminant/substance on soil microorganisms.

Contaminant/ substance	Agroecological practice involved	Behavior of the contaminant/substance in the soil	Ecotoxicological impact on soil microorganisms
Heavy metals[1]			
As, Cd, Cr, Hg, Ni, Pb, Se	**Organic fertilization** (SS, MSW, composts and digestates)	Mobility, bioavailability and toxicity differ according to the chemical speciation (free ionic, complexed, precipitated, oxidation state).	Above a certain threshold, HM are toxic for microorganisms. HM toxicity act primarily at a cellular level, due to the following characteristics: • high affinity for negatively charged cellular groups, such as sulfhydryls, phosphates and hydroxyls; • generation of reactive oxygen species, causing oxidative stress; • competition with essential ions acquisition; • disturbance of cellular ion balance and osmotic regulation. A summary of the literature on metal toxicity to soil microbial processes and populations reveal an **enormous variability in the data**. Two factors contribute to the discrepancies between studies: (1) factors which modify the toxicity/bioavailability of the metals and (2) differences in sensitivity of the microorganism(s) or microbial process(es). Heavy metal concentrations in soils at around current European Union limits have been shown to decrease total microbial biomass, diversity and activity. While most studies focus on the total community, more subtle changes in microbial community structure can also be observed, such as alterations in relative abundance of particular microbial groups or species of agronomical importance. For example, nitrogen-fixing rhizobia are sensitive to metal toxicity. Long-term heavy metal contamination in soil is a selection pressure which can promote bacterial species able to develop **HM resistance**.
Cu, Zn	**Organic fertilization** (livestock manure, SS, MSW, composts and digestates)	**No degradation possible**. Regular application leads to an **accumulation** in the long term (often significant for Cu and Zn). A part of the total metal concentration in soil is irreversibly linked to or sequestered by the soil matrix. **Low solubility** (for consequent low lixiviation). HM concentration in a soil solution is influenced mainly by the soil pH, but also by redox potential, clay content and presence of soil organic matter (SOM). Only a fraction of HMs in solution are **bioavailable** (plants and other biota). It is generally assumed that the free ion is the chemical species which is taken up and causes toxicity when present in excess. Other chemical forms or forms chelated by organic molecules cannot be taken up directly.	
Biological contaminants[2]			
Human and animal pathogens (prions, viruses, bacteria, protozoa, helminths)	**Organic fertilization** (SS, livestock manure, slaughterhouse waste)	Survival times variable, from a few days to multiple years (e.g. <35 to 231 days for *Salmonella*; from <2 weeks to >6 months for enteroviruses). Persistence in the soil is favored by low temperature, high humidity, low light intensity and neutral pH; and by a deep application of OWP.	Interaction with other organisms (predation, competition, antagonism). Poorly characterized.
Antibiotic-resistant bacteria (ARB) and Antibiotic resistance genes (ARG)	**Organic fertilization** (SS, livestock manure, digestates)	The fate of ARBs and ARGs from OWP in soil and their contribution to the overall problem of antibiotic resistance are poorly characterized. Soil bacteria inherently contain ARGs, which makes studies very difficult. Environmental microorganisms are hypothesized to be the main source of antibiotics as well as the concomitant antibiotic resistance. The large numbers of resistant bacteria entering the soil through OWP are likely to compete with other bacteria or survive in the soil environment.	OWP application can increase antibiotic resistance in the soil microflora through several effects: • **horizontal gene transfer** (HGT) of fecal-derived ARGs to native soil microorganisms. HGT mainly includes three pathways mediated by mobile genetic elements, namely extracellular DNA-mediated transformation, plasmid-mediated conjugation, and phage-mediated transduction.

Table 2. Continued

Contaminant/ substance	Agroecological practice involved	Behavior of the contaminant/substance in the soil	Ecotoxicological impact on soil microorganisms
			• **mutation in the native soil microorganisms** through the selection pressure exerted by the residues of antibiotics, metals, PAHs and biocides, causing the appearance of new resistant microorganisms (see section on antibiotics). Although several studies supporting the two concepts have been published, available data are still inconclusive and do not provide direct evidence that links specific factors to individual ARGs.
Trace organic contaminants			
1. Persistent organic pollutants (POP)[3]			
Organochlorine pesticides: aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene **Industrial chemicals**: Hexachlorobenzene, polychlorinated biphenyls (PCBs), Polybrominated diphenyl ethers (PBDE), perfluorinated compound (PFC) **By-products**: hexachlorobenzene (HCB), polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/PCDF), Polycyclic aromatic hydrocarbons (PAHs)	**Organic fertilization (**SS, green manure, crop residues, food residues, MSW,composts, digestates)	• **Persistent**, risk of long-term accumulation in soils. Half-life: years or decades in soil/sediment. **Fates of the pollutants:** • Dissipation from soils by biodegradation and photodegradation (low degradability). Biological decomposition is the most important and effective way to remove these compounds from the environment. • **Binding** to soil solid phases, mainly to SOM but also to the mineral fraction. Pollutant bioavailability decreases with increasing soil-pollutant contact time (= ageing process). • **Transfer to water** (leaching to groundwater and surface water). • Because they are semi-volatile, POPs are transported over long distances in the **atmosphere**. • Transfer to plants and Bioaccumulation.	**Hydrophobic and highly lipid-soluble chemicals**. They accumulate in the membrane bilayer between the acyl chains of fatty acids and increase membrane fluidity. Few studies on the impact of POP on soil microorganisms, even less data on the impact of degradation metabolites. POP exposure might alter the microbial community structure and the metabolic pathways/activities (shown for gut microbiome and pelagic bacterial communities). It has been shown to: • Induce profound changes in bacterial lipid profiles • Disturb bacterial energy metabolism pathways • Disruption in protein export • Induction of bacterial membrane biogenesis • Induction of stress response pathways • Induction of defense of DNA damage
2. Low to medium persistence organic products[4]			
Polydiméthylsiloxane (PDMS), Linear alkylbenzene sulphonates (LAS), phtalates and bisphenols	**Organic fertilization** (SS, MSW, composts digestates)	**Limited data available** on the fate and occurrence of low to medium persistence organic products. Half-life: few days to few years (variable according to the chemical). • **Transformation/degradation** through biodegradation, photodegradation and hydrolysis (principally driven by enzymatic transformations conducted by microorganisms) • **Soil adsorption:** main physicochemical mechanism that prevents leaching or runoff to some extent. Adsorption depends on the chemical, soil properties (including pH, organic matter content, and the concentration and type of divalent cations present), influence of temperature and humidity • **Transport to surface and groundwaters** (leaching and runoff). Dissolved organic matter increase their mobility. • **Transfer to plants**	Variable ecotoxicological impacts on soil organisms, according to the chemical. **Limited data available**. **For antibiotics:** exert a selection pressure on soil microorganisms, conferring antibiotic resistance. Co-exposure to metals, PAHs and biocides increase the appearance of new resistant microorganisms. Antibiotic residues can adversely affect microbial processes in the environment (e.g. nutrient cycling and pollutant degradation).
Pharmaceuticals and personal care products (antibiotics, antidepressants, endocrine disruptors, fragrances, amongst others)	**Organic fertilization** (SS, livestock manure, composts, digestates)		
Some pesticides	**Organic fertilization** (SS, green manure and crop residues, MSW, composts, digestates)		

Table 2. Continued

Contaminant/ substance	Agroecological practice involved	Behavior of the contaminant/substance in the soil	Ecotoxicological impact on soil microorganisms
“Eco-friendly” herbicides[5]			
***β*-triketone herbicides:** sulcotrione, mesotrione and tembotrione	**Crop protection:** weed management	Low mobility in soils. Half-life time of 4 to 144 days depending on soil properties.	No effects on soil microbial diversity and abundance at agronomical dose but some molecule-, dose- and strain-dependent effects at the population level.
Pelargonic acid		Very high to low mobility in soil. Half-life time of 1.6 days.	Ecotoxicological effects on soil microbial communities have not been studied yet.
Simple organic acids: acetic acid		Very high mobility in soil. Half-life time of 0.85 to 1.23 days.	No significant effects on the structure and the diversity of soil microbial communities.
Biopesticides[6]			
Bacillus thuringiensis	**Crop protection:** microbial pesticides	Efficient degradation of Bt proteins in soil. Lack of data concerning the toxicity of the accumulation of some Bt endotoxins in soils.	Limited impact on microbial community structure and microbial diversity in soil.
Trichoderma		**No information available.**	Some studies show an impact of volatiles, toxins and antibiotics produced by Trichoderma on soil microbiome.
Pseudomonas		**No information available.**	Various effects observed, from no prominent alteration of bacterial communities to substantial shift within microbial communities (sometimes suggested as an indirect mode of action).
Spinosad (Saccharopolyspora spinosa)		Relatively fast dissipation of spinosad in soil—Half-life between 1.11 and 2.21 days[7]	Effects on soil enzymatic activities are recorded at high doses or in the short term after application but no negative effects in the long term at the recommended doses of application.
Entomopathogenic fungi		**No information available.**	No or limited adverse effects recorded on soil microbial communities.
Entomopathogenic viruses		**No information available.**	The little studies available tend to show low ecotoxicological risk.
Azadirachtin	**Crop protection:** Botanical pesticides	Low mobility in soil due to its oily composition. No consensus in the literature on its half-life (from a few hours to 8–10 days). Formulated products can have a half-life up to 26 days.[8]	Studies report a toxicity on certain soil microbial groups, somewhat comparable to that observed under the effect of chemical pesticides.
Pyrethroids		Soil bacterial and fungal strains are able to degrade pyrethroids into non-toxic compounds through hydrolysis of ester bond by enzyme esterase/carboxyl esterase.	No observed negative impact to soil microbial community.
Essential oils		Essential oils are known to be easily degraded (mainly by oxidation).	**Effects mostly unknown and poorly described.**
Elicitors, pheromones, allelochemicals, double stranded RNA (dsRNA)-based pesticides and pesticidal substances containing added genetic material	**Crop protection:** Biochemical pesticides, semiochemi-cals and plant incorporated protectants	**No information available**	**Effects mostly unknown and poorly** described but mode of action suggest limited off-target toxicity effects.
Nanopesticides	**Crop protection:** Nanopesticides	**Few studies available** on the behavior in soils. Behavior is depending on the nature of the nanoparticles and of the inorganic nanocarriers.	Some studies tend to show a microbial toxicity of the inorganic nanocarriers.
Mineral pesticides			
Copper	**Crop protection:** mineral pesticide	Mobility, bioavailability and toxicity differ according to the chemical speciation (free ionic, complexed, precipitated, oxidation state). **No degradation possible**. Regular application leads to an **accumulation** in the long term. Please also refer to the heavy metal section.	Negative effects on soil microbial biomass and biodiversity. Please also refer to the heavy metal section.

Table 2. Continued

Contaminant/ substance	**Agroecological practice involved**	**Behavior of the contaminant/substance in the soil**	**Ecotoxicological impact on soil microorganisms**
Microplastics[9] **Coming from the breakdown of biodegradable plastics:** starch-based, polylactide-based or polyhydroxyalkanoate-based	**Crop protection:** weed management (mulching)	**Few studies available:** slight degradation of polylactide-based plastics after 12 months in field conditions[10]	Mainly studied in aquatic environments. Soil studies focus on their biodegradation, not on their ecotoxicological impact.

Acronyms: OWP—organic waste product; SS—sewage sludge; MSW—municipal solid waste; SOM—soil organic matter; HM—heavy metal; ARB—Antibiotic-resistant bacteria; ARG—antibiotic resistance genes; HGT—horizontal gene transfer; MGEs—mobile genetic elements; PAHs—poly-aromatic hydrocarbons; POP—persistent organic pollutants; DDT –; PCB—polychlorinated biphenyls; PBDE—Polybrominated diphenyl ethers; PFC—perfluorinated compound; HCB—hexachlorobenzene; PCDD/PCDF—polychlorinated dibenzo-*p*-dioxins and furans; PAHs—Polycyclic aromatic hydrocarbons; PDMS—Polydiméthylsiloxane; LAS—Linear alkylbenzene sulphonates.

[1](Baath 1989, Giller et al. 1998, Giller et al. 2009, Kupper et al. 2014, Abdu et al. 2017, Barra Caracciolo and Terenzi 2021).[2](Sidhu and Toze 2009, Du and Liu 2012, Martinez 2014, Ghirardini et al. 2020, Ondon et al. 2021, Sanz et al. 2021, Han et al. 2022, Shi et al. 2023).[3](Reid et al. 2000, Brändli et al. 2005, Arias-Estévez et al. 2008, Clarke and Smith 2011, Ren et al. 2018, Rodríguez et al. 2018, Tian et al. 2020).[4](Clarke and Smith 2011, Brandt et al. 2015, Verlicchi and Zambello 2015, Roose-Amsaleg and Laverman 2016, Warner and Flaws 2018; Cycoń et al. 2019, Ondon et al. 2021, Han et al. 2022).[5]EFSA (European Food Safety Authority) 2013; Dumas et al. 2017; EFSA (European Food Safety Authority) 2021a; Thiour-Mauprivez et al. 2022).[6](Mendelsohn et al. 2003, Kookana et al. 2014, Ferraz et al. 2022, Karpouzas et al. 2022, Li et al. 2022, Signorini et al. 2022).[7](Telesiński et al 2015).[8](Ujváry 2010, Kilani-Morakchi S et al. 2021).[9](Serrano-Ruiz et al. 2021, Mo et al. 2023).[10](Slezak et al. 2023).

receiving sewage sludge predominantly contaminated with Zn, a decrease of 7%–11% in soil microbial biomass carbon was observed at concentrations below the UK statutory limit, over a period of 8 years. Similar decreases (7%–12%) were observed in soils receiving sewage sludge predominantly contaminated with Cu. However, soil microbial biomass carbon appeared to show signs of recovery after a period of 6 years. Application of sewage sludge predominantly contaminated with Cd appeared to have no effect on soil microbial biomass carbon at concentrations below the current UK statutory limit.

In addition to the evaluation of chronic and acute toxicity for soil microbes of the contaminants present in the OWP, two important aspects are gaining interest in the scientific community but still need thorough research effort. Firstly, the metabolites from organic contaminant degradation might be as, or more, toxic than their parent molecules, but their consequences on soil microbes are still poorly understood. Secondly, the different contaminants are often studied separately, but they might interact to create a more problematic ecotoxicological impact (cocktail effect). One of the most concerning examples is the co-selection of antibiotic and metal resistance. Long-term heavy metal contamination in soil is a selection pressure that functions as a selective agent in the proliferation of antibiotic resistance (Baker-Austin et al. 2006, Pal et al. 2017, Poole 2017, Nguyen et al. 2019). ARGs and metal resistance genes may be located in the same DNA fragment (Han et al. 2022). In addition to metals, other toxicants contained in some OWP are implicated in the co-selection of antibiotic resistance, including detergents, Poly-Aromatic Hydrocarbons (PAHs) and pesticides (Chapman 2003, Han et al. 2022).

Weed management, tillage and mulching

Soil preparation and weed management strategies go hand in hand. Indeed, tillage helps control weeds by uprooting/burying them and exposing them to unfavorable conditions. However, consequent body of literature revealed the deleterious impact of tillage on soil and its organisms (Karlen et al. 1994, Gómez et al. 1999, Kladivko 2001). Initially developed to reduce soil degradation and production costs, no-tillage (NT) appears challenging because of weed infestation and yield loss, which can lead to the intensive use of herbicides (Colbach and Cordeau 2022). However, it is commonly accepted that the intensive use of synthetic herbicides could have negative impacts on the environment, animals, and human health, and increase weed resistance (Romdhane et al. 2016, Ben Kaab et al. 2020). The strategy choice differs between ecology-based agricultural systems (Table 1). Conservation agriculture is based on no-tillage (NT) or minimum tillage (MT, where soil is not turned over; Bhattacharyya et al. 2022) and a permanent soil cover, but allows the use of synthetic herbicides. In contrast, synthetic herbicides are prohibited in organic farming, and weeds are usually managed by a more intensive tillage. Synthetic herbicides are also prohibited in biodynamic farming, as well as bare tillage year-round, while light tillage is allowed. In agroecology, the principles of input reduction (petrol) and soil health lead to favor MT or NT and direct seeding into living cover crops or mulch. Another way to manage weeds is to use mulches (Daryanto et al. 2018, Somanathan et al. 2022). Traditional mulches are bio-based; made of grass clippings, newspaper, compost, sawdust, dry leaves or bark clipping. A synthetic alternative has been developed in modern agriculture, particularly in nurseries, horticulture and vegetable production: plastic mulch. Low-Density PolyEthylene (LDPE) is the most common type of plastic used in conventional agriculture; at one condition that it must be removed from the field after harvest (Van Schothorst et al. 2021). Ecology-based farming systems are increasingly exploring alternatives to conventional tillage and synthetic herbicide use. Among them, we identified two that might present ecotoxicological risks, and will discuss them in the present section: "eco-friendly" herbicides, authorized in all ecology-based systems, and the use of biodegradable plastic mulch (i.e. corresponding to the standard EN 17033 for the European Union), authorized in organic farming.β-triketone herbicides are derived from leptospermone, a natural phytotoxin produced by the Californian bottlebrush plant *Callistemon citrinus* (Mitchell et al. 2001). These plant protection products (PPPs) were qualified as "eco-friendly" because of their efficiency at low agronomical doses: 350 g.ha^{-1} for sulcotrione and 150 g.ha^{-1} for mesotrione as compared to 1 kg.ha^{-1} for atrazine (Duke et al. 2010, Sidhardhan et al. 2012). However, this "eco-friendly" reputation might be questioned, as these molecules target the 4-HydroxyPhenylPyruvate Dioxygenase (4-HPPD), an enzyme retrieved in plants but also in other organisms, such as mammals or soil bacteria, hence possibly having an effect on non-target organisms (Thiour-Mauprivez et al. 2020a). Recent studies showed no effect on soil microbial communities but a molecule-, dose- and strain-dependent response was demonstrated at the population level, reinforcing concerns about their "eco-friendly" reputation (Thiour-Mauprivez et al. 2020b). Pelargonic acid, a simple fatty acid, is another bio-based herbicide available on the market. Pelargonic acid is a contact herbicide used at high application rates. Its effects are mainly through disruption of the plasma membrane (Dayan and Watson 2011). To our best knowledge, its ecotoxicological effect on soil microbes has not been yet studied. Simple organic acids, such as acetic acid, are also sold for the organic weed control market (Duke et al. 2010). Bottrill et al. (2020) demonstrated in a 7-months field study that neither acetic acid nor its commercial formulation significantly changed the diversity and community structure of soil bacteria and fungi. However, authors noticed local drought conditions that resulted in a rapid degradation of the herbicide and hence modified the exposure scenario of microbial communities to the tested molecules. Various essential oils, such as lemon grass, clove, cinnamon and pine oil, are also considered for weed management (Duke et al. 2010). Some of the components of these oils are interesting because of their unique mode of action. For example, citral, a component of *Citrus aurantiifolia* oil apparently acts by inhibiting single strand DNA-binding proteins (Fagodia et al. 2017, Graña et al. 2020). Here too, there is an urgent need to better study their ecotoxicological impact on soil microorganisms, as many essential oils are known to harbor antimicrobial activities (Habbadi et al. 2018, Kačániová et al. 2020). We will discuss the ecotoxicological aspects of essential oils in more detail in section 4.

Biodegradable plastic mulches (BDM) are commonly made of starch, polylactide (PA) or polyhydroxyalkanoates (PHA) (Miles et al. 2017). As reviewed by Serrano-Ruiz et al. (2021) there are only a few studies addressing the effects of BDM on soil microbial communities. Two recent studies compared the effects of conventional plastic mulch *vs*. BDM. In both studies, the conventional plastic mulch was PolyEthylene-based and the BDM was PLA/PBAT-based (mix of polylactide and polybutylene adipate-*co*-terephthalate). Both studies showed a different response per plastic type. In Reay et al. (2023), microbial nitrogen uptake increased in presence of BDM but decreased in presence of the conventional plastic mulch. In Li et al. (2022), carbon and nitrogen cycling genes abundances were almost systematically responding in the opposite way: when depleted in the bacterial community associated with BDM, they were enriched in the bacterial community associated with conventional plastic mulch. However, genes abundances in the BDM-associated bacterial community were highly different from the ones in the pristine soil, especially nitrogen cycling genes. This was not the case for the conventional plastic mulch-associated bacterial community. Both conventional and biodegradable plastics were degraded, more or less rapidly, in smaller particles called microplastics (MPs) *via* biodegradation, photolysis and hydrolysis (Beltrán-Sanahuja et al. 2021, Somanathan et al. 2022). Some recent studies revealed an effect of MPs on the abundance and the diversity of soil microbial communities. MPs made of PolyPropylene and expanded PolyStyrene were found to create a distinct habitat for bacteria, and induce the recruitment of specific groups, such as Actinobacteria, known as plastic-degraders (Kublik et al. 2022). As a result, clear differences were measured in the community composition of MP-spiked soils compared to the bulk soil. In a pot experiment, PolyEthylene mulch debris were spiked from 0 to 6 g.kg^{-1} and were shown to decrease soil nutrients, proportionally to the dose applied (Liu et al. 2022). The same study also found that bacterial abundance increased but diversity decreased in presence of MPs. Altogether, these results suggest a modification of the bacterial community associated with MPs compared to the one associated with a pristine soil. One can then wonder if these modifications could have consequences on the functions that soil bacteria are supporting and, to a higher extent, on soil ecosystem functioning. Recent studies take an interest in the effect of MPs on bacterial functional genes, especially those involved in nitrogen and carbon cycles, as reviewed by Wang et al. (2022b). However, the effects of MPs seem to be polymer type-dependent. For example, LDPE has been shown to inhibit *nifH* gene expression and promote *amoA* gene expression, whereas PolyVinyl Chloride acts the opposite way (Wang et al. 2022a). It appears then quite difficult to conclude on MP effects on soil microbial communities, as studies focusing on their ecotoxicological impact rather than their biodegradation potential are still recent. In addition, most of the studies still concern MPs coming from the breakdown of conventional plastics and studies on biodegradable MPs are needed (Mo et al. 2023, Table 2).

To conclude, there is no clear regulation or consensus on weed management strategy. The best compromise seems to be the application of a minimal, but necessary, disturbance to the soil in order to keep it functional and minimize the use of PPPs. Mulching can be of interest, but plastic mulches need to be considered with caution. As MP effects on soil microorganisms are yet unpredictable, there is an urge to increase research efforts on terrestrial MP pollution and to develop standardized methods for their analysis in soils, as suggested by Joos and De Tender (2022). Soil microbial community response to multi-stress should also be investigated, as MPs have recently been shown to favor heavy metals and antibiotics sorption, creating a niche where ARGs can be enhanced (He et al. 2022, Syranidou and Kalogerakis 2022).

Pest and disease management

Nowadays, synthetic PPPs have become the foundation of the predominant agricultural systems, and are extensively used to control plant pests and diseases and increase crop yields (Hedlund et al. 2020, Jacquet et al. 2022). However, as already stated before, these agrochemicals have been associated with several human and environmental hazards. The increasing demand for healthy food and environment has greatly enhanced the need for biological pesticides (biopesticides) (Ashaolu et al. 2021, Jacquet et al. 2022). There are no clear guidelines concerning synthetic pesticides in the three fundamental principles of conservation agriculture. However, integrated pest management (IPM) is often recommended. Synthetic pesticides are forbidden in organic and biodynamic systems. They are also in opposition with the principles of soil health, input reduction, biodiversity and self-regulation of agroecology and permaculture. Instead, these four systems recommend the use of biopesticides. Biopesticides can be defined as a biological substance or organism that damages, kills or repels organisms seen as pests or causing a disease. They include pesticides derived from microorganisms (microbial pesticides); naturally occurring substances produced by plants and microorganisms (biochemical or botanical pesticides); semiochemicals (including pheromones and allelochemicals) that are emitted by animals, plants and other organisms; double stranded RNA (dsRNA)-based pesticides and pesticidal substances containing added genetic material (plant-incorporated protectants) (Fenibo et al. 2021, Basnet et al. 2022, Karpouzas et al. 2022). Even if biopesticides, sometimes called "low-risk pesticides", are deemed to be safe due to their biodegradability and natural origin, many scientists agree that they are not devoid of drawbacks. Side effects on non-targeted organisms and/or ecosystem services should be considered (Amichot et al. 2018). However, very few studies address the soil microbial ecotoxicology of biopesticides, and standardized risk assessment tests are not defined yet (Karpouzas et al. 2022).

Microbial pesticides, also called microbial pest control agents (MPCAs), are coming from naturally occurring or genetically altered bacteria, fungi, algae, viruses or protozoans. They also include biological toxin material derived from a microorganism (Usta 2013). Among these, *Bacillus* entomopathogens, especially *Bacillus thuringiensis*, have been used extensively to control insect and fungal pests in crops (*Bt* biopesticides). Even if the obtained results are often inconsistent, the few studies that have been made tend to demonstrate that *Bt* proteins may have a limited impact on soil microbial community structure and diversity (Li et al. 2022). Moreover, the degradation of *Bt* proteins by soil microbes has been widely studied, and appears to be quite efficient (West et al. 1984). However, the lack of toxicity of the accumulation of *Bt* Cry-endotoxins in soils should be further clarified (Mendelsohn et al. 2003). In addition to *Bacillus*, most research and development efforts have focused on two genera: *Trichoderma* and *Pseudomonas*. Studies on *Pseudomonas*, show either no prominent alteration of the bacterial community (Yin et al. 2013, Tienda et al. 2020, Gómez-Lama Cabanás et al. 2022), or a substantial shift within microbial communities for the experimental duration of a couple of weeks (Eltlbany et al. 2019, Elsayed et al. 2020). Obtained results have been suggested to be dependent on the techniques used and the readout parameters. In addition, modulation of the microbiome has been even suggested as an indirect mode of action for some microbial pesticides, *Pseudomonas* included (Berg et al. 2021). *Trichoderma* is producing volatiles, toxins and antibiotics, known to negatively affect the soil microbiome (Jangir et al. 2019). Spinosad is another bacterial-derived insecticide, containing chemical compounds produced by *Saccharopolyspora spinosa*. Studies have shown no negative effects on soil microbiota at the recommended doses of application, while enzymatic activities were negatively affected at higher concentrations (Mohiddin et al. 2015). Other studies point to negative effects in the short term after application, but conclude that spinosad does not pose a long-term threat to the soil environment (Telesiński et al. 2015). Microbial pesticides also include entomopathogenic fungi, like species of the genus *Metarhizium* and *Beauveria*. The concern here is that the release of large quantities of microorganisms devoted to pest control into soil may affect the indigenous soil microbial communities. However, studies conducted on the topic have shown no or limited adverse effects on soil microbial communities (Mayerhofer et al. 2017, 2019; Canfora et al. 2023). Entomopathogenic viruses, such as from the *Baculoviridae* group, are among the least studied biological insecticides (Moscardi et al. 2011) but studies have concluded that they represent a low ecotoxicological risk on soil microorganisms, mainly due to their incapacity to multiply outside its host organism (EFSA (European Food Safety Authority) 2021b). To conclude, it seems that the potential toxicity of microbial pesticides on soil microbiota will depend on their mode of action. Products based on microorganisms which act through parasitism or antagonism have generally no negative impact. It seems however not to be the case for biocidal compounds based on bioactive metabolites produced by microorganisms (Karpouzas et al. 2022).

Botanical pesticides are obtained from plant extracts, essential oils, or a combination (Ahmed et al. 2022). They are naturally occurring chemicals that can act as repellants, attractants, antifeedants, and growth inhibitors (Ngegba et al. 2022). Among them, Azadirachtin, a botanical pesticide produced by the neem tree (*Azadirachta indica* Juss) is widely used to control insects and nematodes (Isman 2006). Studies are reporting contrasted results regarding the effects of this compound on soil microbial communities. Some observe no unacceptable effects on soil microbial functions even at high dose rates (Suciu et al. 2019), some report important non-target organism sensitivity (Felsot and Racke 2006) and unexpected toxicity on certain soil microbial groups, somewhat comparable to that observed under the effect of synthetic pesticides (Singh et al. 2015). Pyrethroids, human-made products of natural pyrethrins derived from the plant *Chrysanthemum cinerariaefolium* are also widely used in fields to control insect pests. It was shown that heavy application of beta-cypermethrin does not cause damage to the soil microbial community (Zhuang et al. 2011). Other studies have identified soil bacterial and fungal strains able to degrade pyrethroids into non-toxic compounds through hydrolysis of ester bond by enzyme esterase/carboxyl esterase (Bhatt et al. 2019), which could explain these observations on soil microbial ecotoxicology. Essential oils also belong to the botanical pesticide category, and their potential as biopesticides is now well established (De Clerck et al. 2020, Ayilara et al. 2023). However, few studies focus on the potentially toxic effects of essential oils on soil organisms, and these effects remain mostly unknown and poorly described (Ferraz et al. 2022).

Among pesticides of mineral origin, copper is certainly the most used one. Copper has been used in agriculture to control oomycetes, fungi and bacteria for over a century. It is essential in organic farming, where disease management depends almost exclusively on its use (La Torre et al. 2018). In particular, the Bordeaux mixture is widely used to control downy mildew of grape since the 19th century. It is the product of reaction of copper sulfate and calcium hydroxide (hydrated lime). There are numerous studies pointing out the negative effect of copper on soil microbial biomass and biodiversity (Shaw et al. 2020, Signorini et al. 2022). The specificity of copper is also its accumulative properties in soil, presenting a risk if used on the long term (Table 1).

Very little, if any, studies have addressed the ecotoxicological impact of biochemical pesticides (semiochemical or elicitor molecules) on soil microbes. Plant-incorporated protectants (PIPs) are biopesticides that are expressed directly in the tissue of genetically modified (GM) crops to protect them from pests (Abdollahdokht et al. 2022). One well known PIP example is the development of transgenic plant incorporated with cry genes from *Bacillus thuringiensis* (*Bt* crops). *Bt* crops effects on soil microbial communities might occur through changes in the quantity and quality of carbon inputs and potential toxic activity of *Bt* protein on soil organisms. Studies tend to show that they are unlikely to cause significant transient or persistent changes in soil microbial communities in the field (Zhaolei et al. 2018, Li et al. 2022). Next-generation double-stranded ribonucleic acid (dsRNA) PIPs are also under development (Fire et al. 1998). As a whole, the environmental fate of macromolecular PIPs remains poorly understood (Parker and Sander 2017). dsRNA-based pesticides have not reached the market yet and no information is available on a possible effect on soil microbiota (Karpouzas et al. 2022). However, the specificity of the mode of action suggests limited off-target toxicity effects.

Nanotechnology is a relatively new method of (bio)pesticide delivery and application that is becoming increasingly relevant.

Nano-pesticides are composed of nanoparticles of less than 100 nm. Their improved stability allows a targeted and controlled release of pesticides, and the use of lower concentrations (Kookana et al. 2014, Abdollahdokht et al. 2022). Nanotechnological approaches have been applied for the development of stable biopesticides with long-term effects. Overall, the small size and high surface/volume ratio of nano-pesticides coupled with the high toxicity of their inorganic active ingredients, open the way to important interactions with soil microorganisms, but also increase the possibility of effects on the soil microbiota. Some studies tend to show microbial toxicity of the inorganic nanocarriers (Zheng et al. 2018, Peixoto et al. 2021). Future studies are needed to assess the effects of these kinds of nano-pesticides on the soil microbiota, comparatively to their conventional formulation and active ingredients (Karpouzas et al. 2022).

Besides the aforementioned products, literature can also be found about artisanal preparations approved at the European level for one or more functions and specific uses. Among these, plant purines (like nettle purine) are the result of anaerobic maceration in water of plants or plant parts (Nasiri et al. 2014). To our knowledge, there are no studies focusing on the impact of these products on soil microbial ecotoxicology. Compost teas, i.e. bio-based extracts originated by mixing mature compost with tap water under controlled condition (Gómez-Lama Cabanás et al. 2022), also enter in this category. Likewise, few studies are available and often show inconsistent results (that could also be explained by great variability of the preparations tested). Because of their ability to reintroduce diverse soil bacteria contributing to nutrient cycling, compost teas were considered in some studies as a way to restore soil bacterial community diversity and promote crop performance under conventional agriculture (Kannangara et al. 2006). However, this positive effect on soil microbiota was not confirmed in recent literature (Bali et al. 2021).

To conclude, we have observed that most of the biopesticides tested seem to present low toxicity and low risk on soil microorganisms when compared to synthetic pesticides. Still, publications often report very contrasted results for some products, and studies are difficult to compare due to the diversity of protocols and measurement units used. Overall, a lot of knowledge gaps remain. An ideal pesticide should not adversely affect organisms other than its targeted pests. Biopesticides are increasingly used to replace synthetic pesticides in pest control, it is consequently necessary to assess their ecotoxicity and especially their non-target effects on soil microorganisms, which is largely unknown, before considering them as a safe alternatives to synthetic pesticides (Table 2).

Considering microbial ecotoxicology to assess the potential of agroecological practices

Overall, agroecological practices have positive impacts on soil microorganisms. This confirms the great potential of agroecology to reach sustainable agriculture. However, we identified some practices that could present ecotoxicological risks for soil microbes (Fig. 1). Organic fertilization can be a source of contaminants, like metals, persistent organic pollutants, antibiotics, etc., if not strictly controlled. Biopesticides can have a deleterious impact on non-targeted microorganisms. Mulching, when done with biodegradable plastic, can lead to MP accumulation in the soil. The different ecology-based farming systems that we considered are characterized by different levels of restrictions with regard to these three practices. In soil conservation farming, the three fundamental principles do not provide specific recommendations concerning fertilization and pest and disease regulation. In organic and biodynamic farming, synthetic pesticides and fertilizers are forbidden. Fertilization is based on organic matter inputs (and naturally occurring mineral fertilizers), whose quality is controlled, and crop protection based on biopesticides and natural pest and disease regulation. The use of synthetic fertilizers and pesticides is not aligned with the principles of soil health, input reduction, biodiversity and self-regulation of agroecology and permaculture. Biodegradable plastic mulch is allowed in organic agriculture, but there are no clear guidelines on this practice in the other ecology-based systems. At a global scale, a recent meta-analysis (Christel et al. 2021) showed that the strictest ecology-based systems are the most beneficial for soil microbes. Biodynamic farming appears as the farming systems with the most favorable effect on the soil ecological quality, with 70% and 52% of the biological indicators measured higher than in conventional and organic farming, respectively. Organic farming ranks second, with 69% of biological parameters higher than in conventional farming. Soil conservation farming would rank third since 57% of biological indicators show a more positive effect than conventional farming does. In this study, organic fertilization and longer crop rotations were pointed out as the most favorable practices, whereas the use of pesticides and soil tillage were cited as the most deleterious ones. The impact of agroecological and permacultural systems on soil microorganisms are still poorly documented. As they are based on a set of principles, and not on the specifications of a standard, they leave room for different interpretations and lead to multiple possible combinations of practices, hence the difficulty to assess them. The great strength of these principle-based farming systems is their integration of farmers and farming communities. Especially agroecology, whose multiple dimensions encompass culture, economic structure, social justice, food security and sovereignty, environment, food policy, research, governance (Gliessman et al. 2022). Another advantage of agroecology is that its principles are purposefully broad and adaptable to various contexts. Most of the other modern ecology-based farming systems were developed mainly in Europe and America, and there is a massive lack of research evidence regarding the adequation of their standards for other continents.

Because of the ecotoxicological risks of misuse of ecology-based farming practices, there is a need to set guidelines at the national and multinational level, in parallel to the on-farm regulations (requirements for a certification), for the use of different products (organic fertilizers, biopesticides and biodegradable mulch), as well as critical threshold values in soils. To do so, the ecotoxicological impacts on soil microorganisms of these products still need to be thoroughly evaluated on microbial communities in agricultural fields. The first reason is the high variability of intrinsic characteristics, especially for OWP and artisanal biopesticide preparations (origin, mixture, treatment, contaminants concentration, stability, maturity, etc.). Secondly, the beneficial or adverse effects of substances on agricultural ecosystem depend on many different factors such as soil type and properties, cropping system or climatic conditions (Urra et al. 2019). Thirdly, studies vary widely in terms of application quantity and frequency, duration of trial, experimental conditions (field *vs.* greenhouse *vs.* lab studies), and time between application and sampling. Lastly, the metrics used to characterize microbial communities (e.g. qPCR, amplicon sequencing, shotgun metagenome) are not standardized and all have limitations which

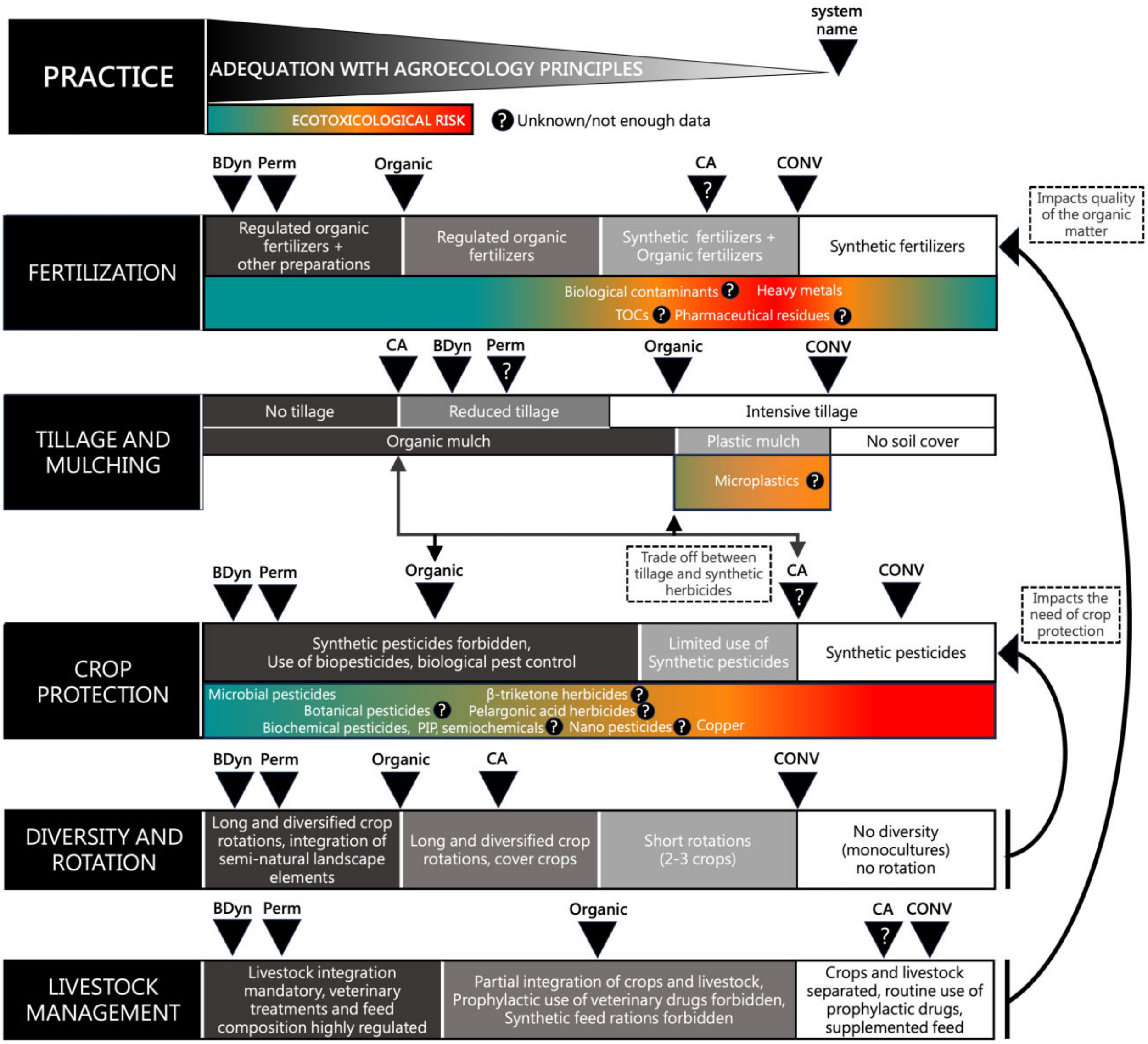

Figure 1. Graphical synthesis of the present review. For each of the five main practice categories, the sub-practice categories are classified according to a gradient of increasing adequation with agroecology principles. On top of the sub-practice categories, triangles indicate the position of the agrosystems in the gradient: (1) Biodynamic system (BDyn); (2) Permaculture (Perm); (3) Organic farming (Organic); (4) Conservation agriculture (CA); (5) Conventional agriculture (Conv). Please note that the position of the marker is approximate, especially for the systems based on a set of principles and not on the specifications of a standard. An interrogation point in the marker indicates that the practice is not taken into account in the standards or principles. Below the sub-practice categories, the ecotoxicological risk is presented in a gradient from green (low risk) to red (high risk), and the involved substances are located in the gradient. When the ecotoxicological risk is still unknown or poorly documented, an interrogation mark accompanies the substance/category of product.

may hamper the assessment of ecotoxicological effects. The effect might be different for biomass measurements *vs.* diversity metrics and at the community *vs.* the population level. Nucleic acid-based approaches are still widely used when working on microbial ecotoxicology and comparing microbial diversity between different environmental samples is not an easy task (Hellal et al. 2023). There is an urgent need to develop standardized methods based on functions with in vitro tests focusing on ecologically-relevant microbial groups (i.e. AMF or ammonia-oxidizing microbes; Karpouzas et al. 2022) to better understand their roles in such a complex environment (Hellal et al. 2023). In parallel, more efforts have to be done on the definition of what a healthy soil microbiome is, in order to set up clear indicators. However, one should be aware that risk assessments made at the microbiome level could render costlier alternatives to synthetic pesticides, slowing down their arrival on the market, at the expense of farmers. Another challenge is the underutilization of important sources of organic matter and nutrients resources, because of their contaminant content. As more and more of this material is produced off farms, the most important and also best strategy, is to reduce the contaminant concentration of these OWP by, among others, working on source separation of wastes (for MSW, composts, digestates), reduction of pharmaceutical use for humans and other animals (for manures, sewage sludges, etc…), and the development of new treatments (improved digestion and composting processes, etc…).

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Marie-Liesse Vermeire (Conceptualization, Writing – original draft, Writing – review & editing), Clémence Thiour-Mauprivez (Conceptualization, Writing – original draft, Writing – review &

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