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## **Improved assessment of the impacts of plant protection products on certain soil ecosystem services requires better consideration of terrestrial microalgae and cyanobacteria**

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### **Abstract:**

There is growing scientific and societal consciousness that the environmental risks and impacts of plant protection products (PPPs) cannot be properly assessed without considering ecosystem services. However, the science on this issue remains incomplete and fragmented, as recently illustrated in a collective scientific assessment that pointed out the limited knowledge on the risks and impacts of PPPs on soil ecosystem services, which are clearly overlooked. Beside soil ecosystem services, certain key players involved in these services are largely overlooked in the scientific literature on the risks and impacts of PPPs, namely soil microbial photosynthetic communities. Here, we followed the principles of evidence-based logic chain approaches to show the importance of considering these microorganisms when studying the impacts of PPPs on certain services provided by soil ecosystems, with a focus on regulating and maintenance services that play a role in the regulation of baseline flows and extreme events. Terrestrial microalgae and cyanobacteria are ubiquitous photosynthetic

microorganisms that, together with other soil micro- and macro-organisms, play key roles in the ecosystem functions that underpin these ecosystem services. There is an extensive literature on the ecotoxicological effects of PPPs on different organisms including soil microorganisms, but studies concerning soil microbial photosynthetic communities are very scarce. However, there is scientific evidence that herbicides can have both direct and indirect impacts on these microbial photosynthetic communities. Given that they play key functional roles, we argue that soil microbial photosynthetic communities warrant greater attention in efforts to assess the environmental risks and impacts of PPPs and, ultimately, help preserve or restore the regulating and maintenance services provided by soil ecosystems.

#### **Effects of PPPs on the regulating and maintenance services provided by soils**

Prompted by recommendations made by the European Food Safety Authority (EFSA 2010; 2016), scientists, environmental managers, stakeholders, and regulators are increasingly conscious of the importance of protecting ecosystem services against the adverse effects of plant protection products (PPPs) (Nienstedt et al. 2012; Arts et al. 2015; Rumschlag et al. 2020). Several scientists in the fields of ecotoxicology and environmental risk assessment of chemicals have been working for years to develop research and propose conceptual and operational approaches to achieve this objective (e.g., Cairns and Niederlehner 1994; Forbes and Calow 2013; Brown et al. 2021; Faber et al. 2021; Maltby et al. 2022). However, there are still few studies providing hard information on the risks and effects of PPPs on ecosystem services, and the resulting science on this issue remains incomplete and fragmented (Pesce et al. 2023a). This was highlighted by the conclusions of a collective scientific assessment performed between 2020 and 2022 addressing the state of knowledge on the effects of PPPs on biodiversity and ecosystem services (Pesce et al. 2021). In particular, this collective scientific assessment pointed out the limited knowledge on the effects of PPP on services provided by soil ecosystems (Pesce et al. 2023b). According to the most recent version of the Common International Classification of Ecosystem Services (CICES, version 5.1.; Haines-Young and Potschin 2018), these services include regulating and maintenance services belonging to the group "regulation of baseline flows and extreme events," especially the classes "control of erosion rates," "buffering and attenuation of mass movement," and "hydrological cycle and water flow regulation, including flood control and coastal protection," which are largely neglected in the scientific literature dealing with the impacts of PPPs.

## **Abundance, diversity, and functional role of soil microbial photosynthetic communities in soil ecosystems**

Terrestrial microalgae and cyanobacteria communities (mainly designated hereafter as soil microbial photosynthetic communities) are ubiquitous photosynthetic microorganisms. They are pioneer autotrophic components of microbial communities growing on the surface of soils (Fig. 1). In temperate agricultural soils, they are highly abundant (from  $1 \times 104$  to  $1 \times 107$  cells/g soil, depending on season and land use, (Metting 1981; Zancan et al. 2006). Soil microbial phototroph communities can reach for around 25% of the microbial biomass of agricultural soils (Abinandan et al. 2019). These communities are highly diversified: eukaryotic microalgae are mainly represented by Chlorophyceae, Bacillariophyceae (Foets et al. 2021), Xanthophyceae and Chrysophyceae, while prokaryotic cyanobacteria include Oscillatoriales, Nostocales, and Chroococcales, many of which are filamentous (Metting 1981; Davies et al. 2013; Lentendu et al. 2014; Djemiel et al. 2020). While certain groups are thought to be specific to soils, others are also found in aquatic environments (Bérard et al. 2004), suggesting biological transfers between aquatic and terrestrial environments (Pfister et al. 2017).

In some crops alternating between flooding and drying out (e.g., rice paddies),cyanobacteria, and, to a much lesser extent, microalgae have been deeply studied on the surface of emerged and submerged soil (Singh et al. 2018; Kaushik et al. 2019). Soil microbial photosynthetic communities form a soil– atmosphere microbial interface (subjected to sprayed PPP), which makes them central to addressing the challenges of sustainable agriculture and preservation and management of ecosystem services, in particular soil health and agricultural production (Ramakrishnan et al. 2023).

*Fig. 1: Pictures illustrating soil microbial phototrophs. a Conventional cropland (maize), b conservation cropland (barley), and c forest (Mediterranean). Microscopy observations: d diatoms; e cyanobacteria; f and g photosynthetic microbial biofilms growing in 50 days incubated microcosms on soil aggregates from organic cropping system: incubation with the herbicide isoproturon (g) at field dose recommended as 2.4 L ha−1 of the commercial formulation clearly inhibited the development of photosynthetic biofilms on soil aggregates, compared with incubated microcosms without isoproturon (f)* 



Indeed, these microbial communities are involved in many ecosystem functions, which are achieved via various ecological processes (Fig. 2), such as photosynthesis, pigment production, biofilm/filament, and exopolysaccharide production (Malam Issa et al. 2007; Chamizo et al. 2018), enzymes, hormone and biocide production (Abinandan et al. 2019; Poveda 2021; Santini et al. 2021; Righini et al. 2022; Osman et al. 2023), and N2 fixation (Peng and Bruns 2019). In croplands that experience frequent soil surface disturbances (e.g., tillage practices), microbial production by soil photosynthetic microorganisms can range from 80 to 157 g C m−2 year−1, which represent 6 to 7% of the net primary production of terrestrial vegetation (Shimmel and Darley 1985; Jassey et al. 2022). The presence of soil microbial photosynthetic communities has also been shown to reduce the net emission of CO2 from agricultural soils under experimental conditions (Sauze et al. 2017).

Experimental and field studies have already demonstrated that soil microbial photosynthetic communities play a role in maintaining soil structure by producing exopolysaccharides and filaments/biofilms that help to control (i) soil aggregation and thus limiting erosion (Metting 1987; Knapen et al. 2007; Kidron and Drahorad 2021; Keqiang et al. 2023) and (ii) soil water retention that contributes to hydrological cycle and water flow regulation (Cantón et al. 2020; Rossi et al. 2021; Giora et al. 2020; Kidron 2021). Native soil microbial photosynthetic communities can also enhance soil dissipation of some PPPs, suggesting a key role on soil surface as a bio-filter (Davies et al. 2013). The inoculation of biocrust organisms (particularly pioneer cyanobacteria) is increasingly used as a soil restoration solution (e.g., Chamizo et al. 2020; Zhao et al. 2021, Dhawi 2023). However, in the context of agroecosystems, the knowledge about the functional role of soil microbial photosynthetic communities is scarce and mostly outdated (Metting 1987; Knapen et al. 2007; Malam Issa et al. 2007; Peng and Bruns 2019), with the exception of rice fields, where microalgae and cyanobacteria are recognized for their beneficial effects on crops and are sometimes taken into account in rice cropping system simulations (Gaydon et al. 2012).

*Fig. 2: Putative list of ecological processes performed by terrestrial photosynthetic microorganisms in soil ecosystems and possible unweighted contribution of each of these processes to soil ecosystem functions classified according to the categories proposed by Pesce et al. (2023a)* 



Given the diversity of ecological processes performed by soil microbial photosynthetic communities, they may be significantly involved in many of the 12 categories of ecosystem functions potentially impacted by PPPs, as recently proposed by Pesce et al. (2023a). Ultimately, these ecosystem functions are the pillars of numerous soil ecosystem services. This is why we call for better consideration of soil microbial photosynthetic communities to preserve or restore soil ecosystem services.

#### **Limited knowledge about the effects of PPPs on terrestrial microalgae and cyanobacteria**

There is an extensive literature on the ecotoxicological effects of PPPs on different taxonomic groups (including for instance terrestrial macro-invertebrates and heterotrophic microorganisms as well as aquatic microalgae and cyanobacteria), but soil microbial photosynthetic communities remain some of the least documented biological communities in ecotoxicology. However, a few studies have shown that pesticides can negatively impact these non-target communities (when considering the community level, less than 30 relevant articles were obtained from the bibliographic analysis performed on the Web of Science — WoS). For instance, herbicides have been shown to strongly affect these terrestrial photosynthetic microbial communities by decreasing their biomass and diversity (Pipe 1992; Zancan et al. 2006; Crouzet et al. 2013; Joly et al. 2014). Herbicide effects also disrupt ecological processes such as soil photosynthesis (Bérard et al. 2004), biomass production (Pipe 1992), N2 fixation (Dash et al. 2018; Wegener et al. 1985), and pigment and exopolysaccharide production (Crouzet et al. 2019; Joly et al. 2014; Zaady et al. 2013). Such effects can have further consequences on ecosystem functions. Zaady et al. (2013) reported that the triazine herbicide simazine increased soil runoff and decreased hydraulic conductivity due to its negative impact on soil chlorophyll biomass and exopolysaccharides. Crouzet et al. (2019) showed that the phenylurea herbicide isoproturon has a negative impact on soil aggregate stability by decreasing chlorophyll biomass and exopolysaccharide production and changing pigment composition.

### **Potential impacts of PPPs on soil ecosystem services: "regulation of baseline flows and extreme events" as an example**

As mentioned in the previous section, scientific evidence exists concerning the impact of some herbicides on several ecological processes performed by soil microbial photosynthetic communities, including photosynthesis, pigment, EPS, and possibly filament production (components of filamentous microalgae and cyanobacteria biomass impacted by herbicides), as well as N2 fixation. Herbicide effects on such ecological processes can have consequences on different ecosystem functions which themselves support soil ecosystem services.

*Fig. 3: Schematic network of logic chains showing how three classes of the "Regulation of baseline flows and extreme events" ecosystem services and related goods and benefits can suffer cascading effects caused by ecotoxicological impacts of herbicides on selected ecological processes carried out by soil microbial phototrophs. EPS, exopolysaccharides excreted by microbial phototrophs in the soil* 



To improve the assessment of the risks and effects of PPPs (or other chemicals) on ecosystem services, several authors advocate the development of evidence-based logic chain approaches (Hayes et al. 2018; Faber et al. 2021; Maltby et al. 2021).

These approaches use logic chains on specific impacts, from effects on key lower-trophic-level organisms, which disrupt a wide range of ecological processes and functions, to consequences on broader ecosystem services (Hayes et al. 2018). In Fig. 3, this kind of approach was used to illustrate how ecotoxicological effects of PPPs on the abovementioned ecological processes performed by soil microbial photosynthetic communities can impact some ecosystem functions supporting soil ecosystem services belonging to the group "regulation of baseline flows and extreme events" and on associated goods and benefits (CICES, version 5.1.; Haines-Young and Potschin 2018).

This specific example, which shows how some regulation and maintenance services provided by soil ecosystems can suffer cascading effects caused by ecotoxicological impacts of PPPs on ecological processes carried out by soil microbial photosynthetic communities, highlights the need to consider these organisms in environmental assessments on the risks and effects of PPPs (and other chemicals), not only in order to protect these communities, their diversity, and the ecological processes they perform, but also to protect or restore the functioning of soil agroecosystems and the services they provide, as addressed in semi-arid contexts with biocrusts (Zhao et al. 2021). However, risk and effect assessment targeting more specifically selected soil ecosystem functions and services needs taking into consideration all the main biological actors involved, meaning that soil phototrophic microorganisms should be studied together with other soil microorganisms identified as key interacting players of a soil microbial ecosystem as well as macro-organisms identified as key players in the considered evidence-based logic chain.

#### **Conclusions and recommendations**

Very few studies have been carried out to characterize the risks and impacts of PPPs on the diversity and activities of soil photosynthetic microbial communities, and most were published in the 1970s– 1990s, which explains the paucity of quantitative data on these effects (Mamy et al. 2022). Current research on this topic mainly concerns rice fields, with a focus on cyanobacteria and their diazotrophic activity contributing to N2 fixation (Kaushik et al. 2019). In order to significantly develop knowledge concerning the impact of PPPs on soil microbial photosynthetic communities, it is necessary to consolidate the methods for sampling and characterizing these communities in different soils and land use contexts (Barragan et al. 2018). Besides these methods, which have to be specific to the terrestrial environment, it is also important to draw on methodological and conceptual developments from research aimed at assessing the impact of PPPs on freshwater photosynthetic microorganisms, which are the subject of numerous studies at various biological levels (Vonk and Kraak 2020). For example, the use of functional traits, as applied to aquatic microalgae (Baert et al. 2017), could help to better understand the functional consequence of the ecotoxicological effects of PPPs on soil microbial photosynthetic communities, calling for further investigations about their own functional traits. The application of omics approaches (such as metabarcoding, Djemiel et al. 2020; Rivera et al. 2020; as well as transcriptomics, proteomics and metabolomics, Moisset et al. 2015, Lips et al. 2022), which are increasingly used to investigate the structural and functional responses of aquatic photosynthetic microorganisms to PPPs at different biological levels (from strains to communities) could provide new insights into the effects of PPPs on soil photosynthetic microorganisms. For instance, it could help to investigate their sensitivity to PPPs, based on their phylogeny, and to study alterations in community diversity and functions in response to PPP exposure. Fluorimetry techniques (PAM and spectral measurements), currently applied to aquatic environments, could also be used to characterize the responses of soil photosynthetic microorganisms to PPPs. Promisingly, it has thus been shown that spectral analyses based on reflectance or fluorescence can be applied to the study of soil biological crusts (Rodriguez-Caballero et al. 2015).

Flow cytometry, which is already widely used in aquatic environments, can also be applied to soil ecosystems (Menyhart et al. 2018). Finally, the development of imaging tools (Zhang et al. 2022, 2023) would also enable progress to be made in taxonomic recognition and more global observation on the scale of microbial crusts.

Moreover, relying on these different methods, terrestrial photosynthetic microbial communities could potentially serve as good indicators of soil quality. The biodiversity of freshwater diatoms is currently used for biomonitoring purposes, to assess the ecological quality of aquatic ecosystems, thanks to the use of biological indices (e.g., BDI, Coste et al. 2009) based on the large ecological amplitude of diatoms and their species-specific sensitivity to pollution. The connectivity between terrestrial and aquatic environments means that several photosynthetic microorganisms can be found both in soils and waters. One example is the taxonomic group of diatoms. Martínez-Carreras et al. (2015) and Klaus et al. (2015) demonstrated rapid transfers of terrestrial diatoms from soil surfaces to surrounding waters. The diatom group may therefore hold potential for use as an indicator of pollution (especially PPPs) throughout the terrestrial–aquatic continuum.

In general, there is a need to make progress on the links between diversity, function, and services impacted by PPPs (Pesce et al. 2023a). Going beyond the simple question of PPPs, this is clearly identified as a major challenge in microbial ecotoxicology (Hellal et al. 2023). Thus, efforts to improve the evaluation and quantification of the impacts of PPPs and other toxicants on soil ecosystem services demand greater interdisciplinarity, with more interaction between ecotoxicologists, environmental scientists in other disciplines, such as those that connect to physics and pedology (in order to take into account the specific context of soils) and scientists from the humanities and social sciences working in the field of ecosystem services. This nexus of sciences and disciplines will be an essential step towards sustainable use of PPPs that can help maintain or even restore the ecological quality and functioning of soil ecosystems and thus enable them to provide vital ecosystem services.

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