

Assessment of the impact of pesticides on the soil microbial community using intact polar membrane lipids as biomarkers by HPLC ESI-MSⁿ

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1 INTRODUCTION

- The impact of pesticides and their transformation products on soil health is an emerging issue.
- The impacts of pesticides on the distribution, structure and function of soil microbial communities can disturb the soil fertility.
- Soil microbes play a vital role in carbon and nitrogen cycles (Fig1A).
- Analysis of intact polar membrane lipids (IPLs) (Fig. 1B,C) can be a powerful tool to investigate viability and diversity in microbial populations and can be used to trace changes in the microbial communities in soil (Rütters et al., 2002).
- liquid chromatography mass spectrometry can be used for the analysis of trace levels of IPLs (Zink et al., 2008).
- The present study was focused on assessment of the impact of isoproturon (IPU), monodesmethyl isoproturon (MDIPU), didemethylisoproturon glyphosate (GLYP), aminomethyl phosphonic acid (AMPA), thifensulfuron methyl (TSM) and propyzamide (PROP) as potential stressors, on the IPLs of the soil microbial community.

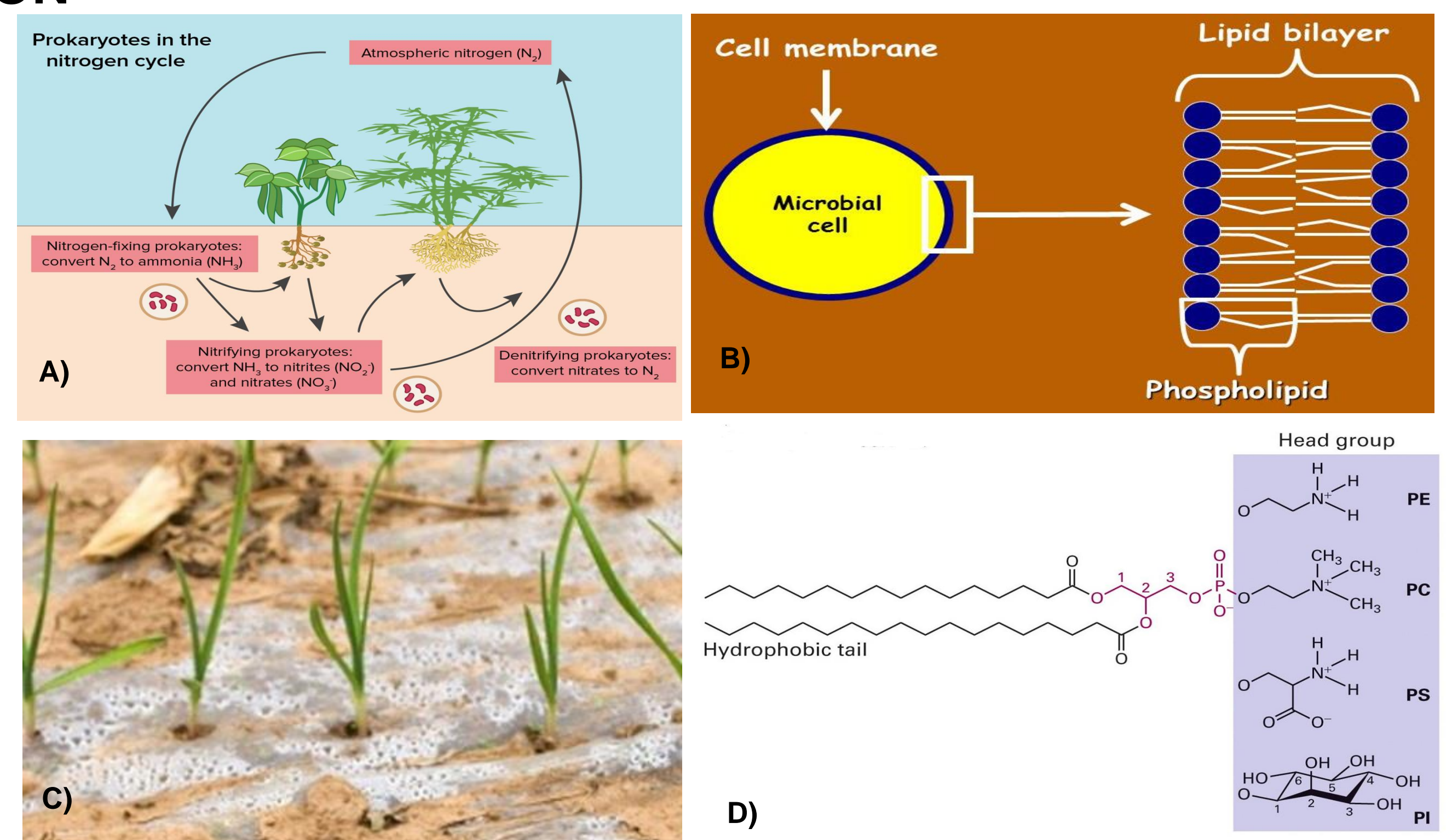
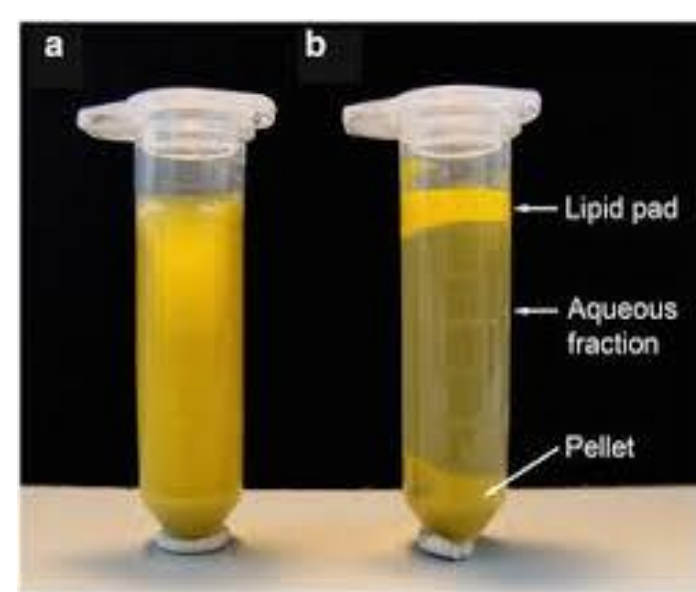


Fig.1: Role of soil microorganisms in nitrogen cycle (A), Cell membrane: a lipid bilayer (B), Cultivated field affected due to pesticide application (C), : membrane lipids with polar head groups

2 EXPERIMENTAL



Soil sample collected from uncultivated field was filled in plastic pots and separately treated with IPU, MDIUP, DDIPU, GLYP, AMPA, PROP, and TSM at 2X recommended application rates.



IPLs were extracted from the pesticide-treated and control soils after 49 days by modified Bligh Dyer method (1959). Polar lipids fractions were obtained by separation via flash column chromatography using activated silica.



Methanol fraction of IPLs of each of the treated and control samples were run on HPLC-ESI-MS/MS. IPLs were detected and confirmed via their retention times and MS fragmentation pattern on the basis of their polar head groups

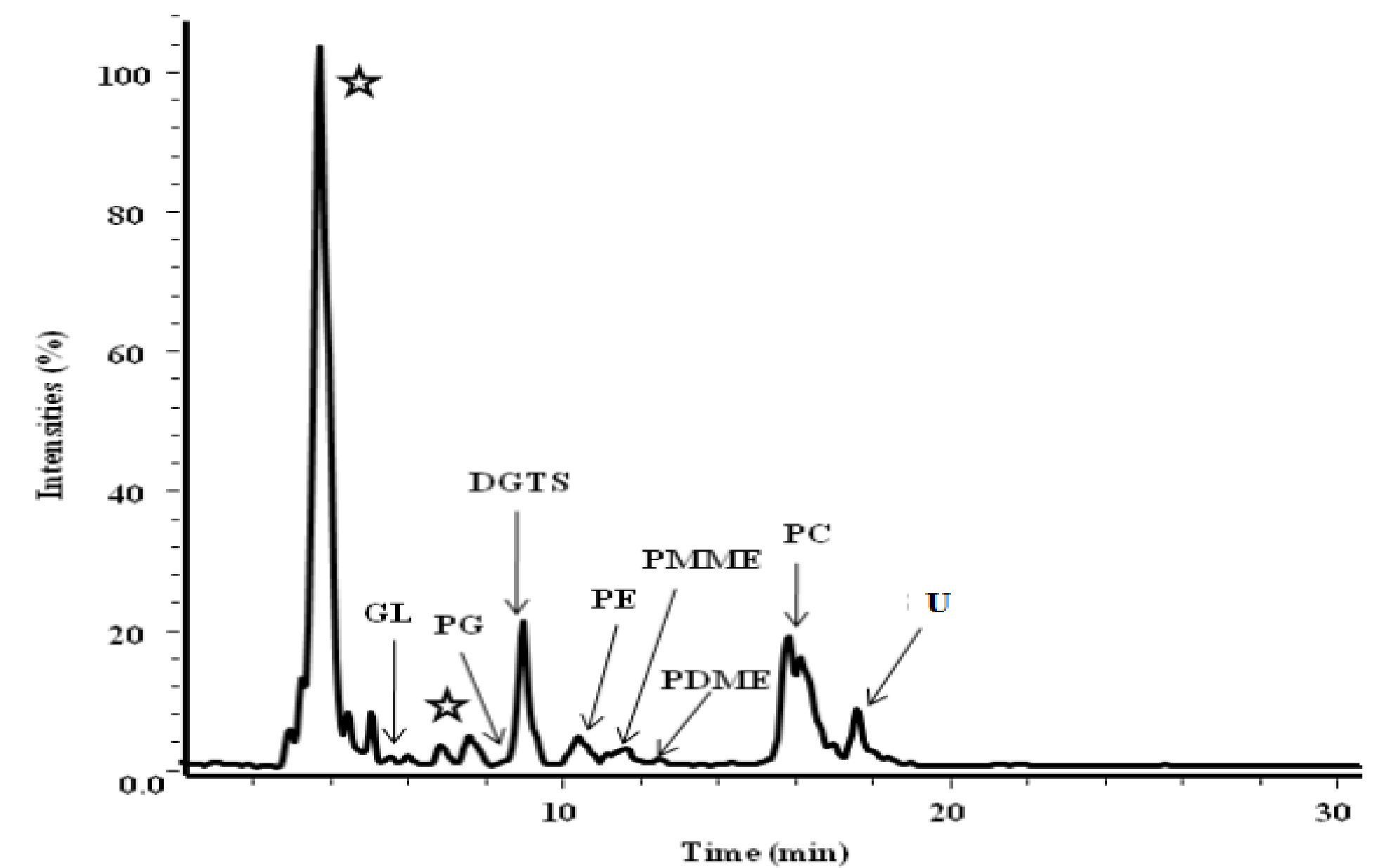


Fig2: LCMS/MS Chromatogram of IPLs extracted from the pesticide treated and control soil samples.

GL; glycolipids, **PG**; diacylphosphatidyl glycerol, **DGTS**; diacylglyceroltrimethyl homoserine, **PC**; phosphatidyl choline, **PE**; phosphatidyl ethanolamine, **MMPE**; monomethyl phosphatidyl ethanolamine, **DMPE**; dimethyl phosphatidyl ethanolamine, **U**; unknown lipid, **IPU**

3 RESULTS AND DISCUSSION

- The IPL profiles of IPU, GLYP and PROP treated soil showed (Fig3A) pronounced variations among the abundance of the polar lipids especially for diacylglycerol trimethylhomoserine (DGTS).
- The impact of the pesticides was more obvious in changes induced in the fatty acyl compositions of phosphatidyl ethanolamine (PE), monomethylphosphatidyl ethanolamine (PMME), monogalactosyl diacylglycerol (GL) and phosphatidyl glycerol (PG).
- The greatest changes were observed among the PE, PMME, PG and MGDG molecular species (fatty acids composition), while minimal variations were found for the PC and DGTS classes of lipids.
- Membrane lipids including phospholipids and their fatty acids side chains often give evidence of adaptations by showing for instance, response to changing nutrient availability, temperature, pressure or any other environmental stressor (Laura et al, 2010)
- The adaptation process might be associated with the changes in fatty acids carbon numbers and their saturation or unsaturation.
- It was concluded that the botanical pesticides and their TPs can induce changes in soil microbial diversity.
- Moreover, IPLs can be used as biomarkers for the assessment of the microbial response toward environmental stressors.

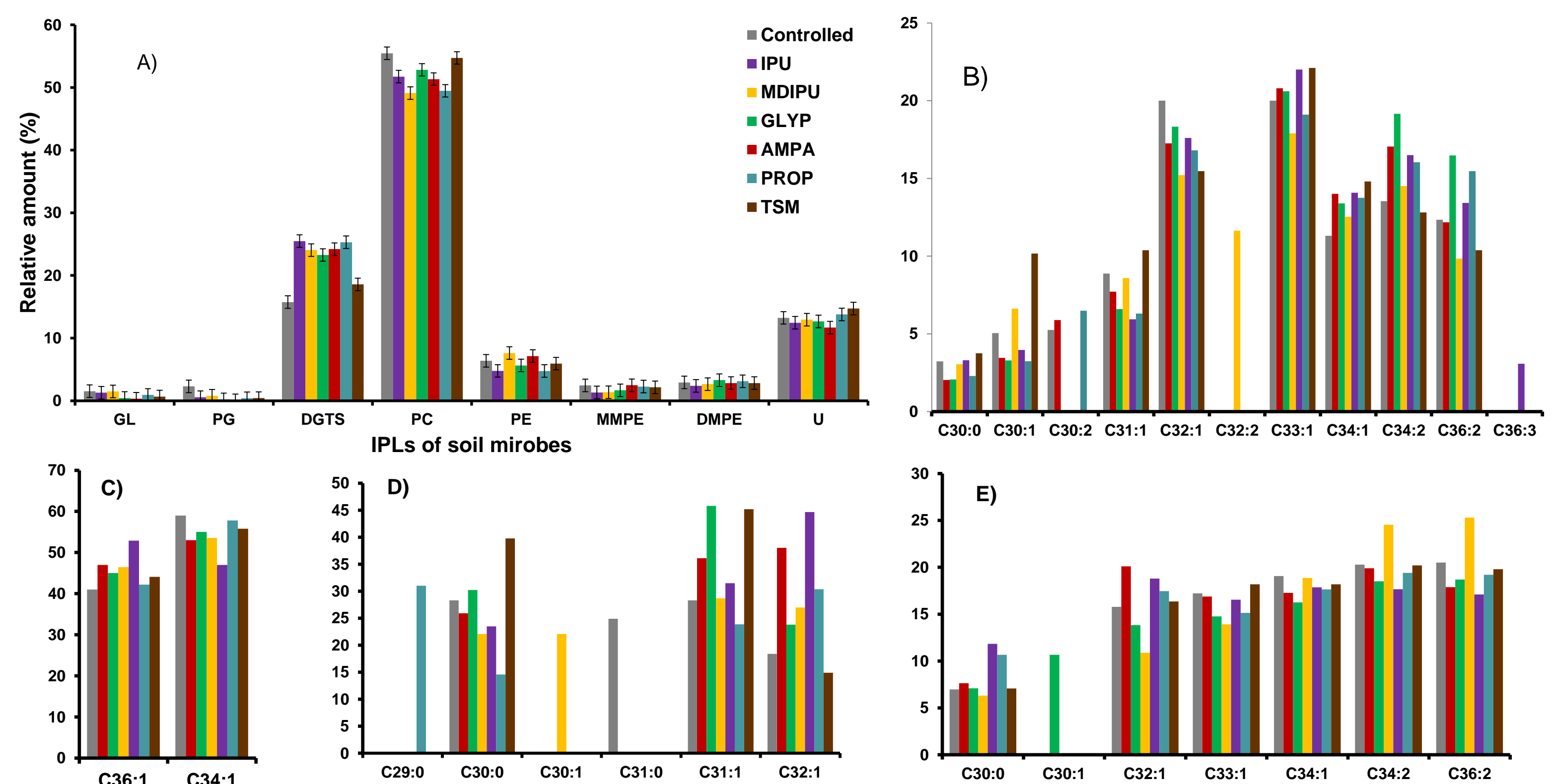


Fig.3 :Relative intensities of IPLs (% area) of controlled and pesticides-treated soils (A). Relative intensities (%) of the molecular species of control and pesticides treated soils; PE (B), MGDG (C), PMME (D), PDME (E).

4 REFERENCES

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