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Monitoring the effects of neonicotinoid pesticides on the phyllosphere and soil bacterial communities in a three-year soybean and corn rotation Corn

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OBJECTIVES

- **Characterize** soybean and corn phyllosphere and soil **bacterial community composition**
- **Determine the bacterial variation and temporal dynamics**
  - **In a three-year** soybean/corn crop **rotation**
  - **In response to neonicotinoid (neonic)** seed treatment

METHODS

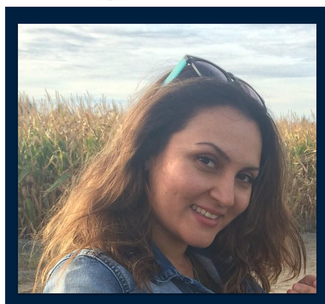
- **Collecting samples**
  - **Phyllosphere: 50-100g** per sample of the plants' middle **leaves** (**144 samples** in total) (see Table 2)
  - **Soil: 400-500g** per sample of **bulk soil** from the upper 12-15 cm layer of soil and at 10 cm from the sampled plants (**192 samples** in total) (see Table 2)
- **Extracting bacterial DNA,** using PowerSoil DNA isolation kit
- **Amplifying** the hypervariable **V5-V6 regions of 16S rRNA gene,** using chloroplast-excluding 799F-1115R primers
- **Sequencing DNA amplicons,** using Illumina MiSeq
- **Processing data into** an **amplicon sequence variants (ASVs)** table for each sample and **assigning taxonomy,** using **DADA2**
- **Analyzing data** using R packages

RESULTS

- **Plant host species** explained **12% (phyllosphere) and 2% (soil)** of the bacterial diversity.
- **Host growth stage** (= **month** in figure 2) was the strongest driver of the **phyllosphere** bacterial composition diversity and explained **more than 20%** of the soybean and corn phyllosphere bacterial variation.
- **Year** also explained the bacterial diversity in **soybean phyllosphere (11%) and soil (5%).**
- **Neonic** had an impact on the phyllosphere and especially soil bacterial community composition, **individually and in interaction with host species, host growth stage and time (month and year).**
- The effects of **neonic on soil** bacteria **increased during growing season** (July and August) and then **decreased towards the end of the season** (September).

CONCLUSIONS

- **Plant host (species and growth stages) and time (month and year)** are **stronger drivers** of variation in phyllosphere bacterial composition than neonic application; however, **Neonicotinoids in interaction** with this parameters influence the **phyllosphere** bacterial diversity.
- **Neonicotinoid pesticides** have an impact on the **soil** bacterial taxonomic composition. These impacts change over time (months and years) and are more significant in the **middle of the growing season.**



Neonicotinoid pesticides in interaction with **plant host** (species and growth stage) and **time** (month and year) influence **phyllosphere and soil bacterial diversity.**

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Neonicotinoids have an impact on the **soil taxonomic composition**, especially in the **middle of the growing season.**

	Phyllosphere		Soil	
Variables	**R2(%)**	**Pr(>F)**	**R2(%)**	**Pr(>F)**
Host	12	0.001	2	0.001
Year	8	0.001	3	0.001
Month/Growth stage	14	0.001	3	0.001
Neonic	1	0.003	3	0.001
Host:Neonic	1	0.001	1	0.004
Year:Neonic	1	0.010	1	0.006
Month:Neonic	1	0.029	2	0.031
Host:Month:Neonic	1	0.019	-	-
Year:Month:Neonic	1	0.019	-	-
Soybean	**Phyllosphere**		**Soil**	
Variables	**R2(%)**	**Pr(>F)**	**R2(%)**	**Pr(>F)**
Year	11	0.001	5	0.001
Month/Growth stage	22	0.001	5	0.001
Neonic	1	0.023	4	0.001
Year:Neonic	1	0.033	2	0.005
Corn	**Phyllosphere**		**Soil**	
Variables	**R2(%)**	**Pr(>F)**	**R2(%)**	**Pr(>F)**
Month/Growth stage	27	0.001	9	0.001
Neonic	6	0.001	4	0.001

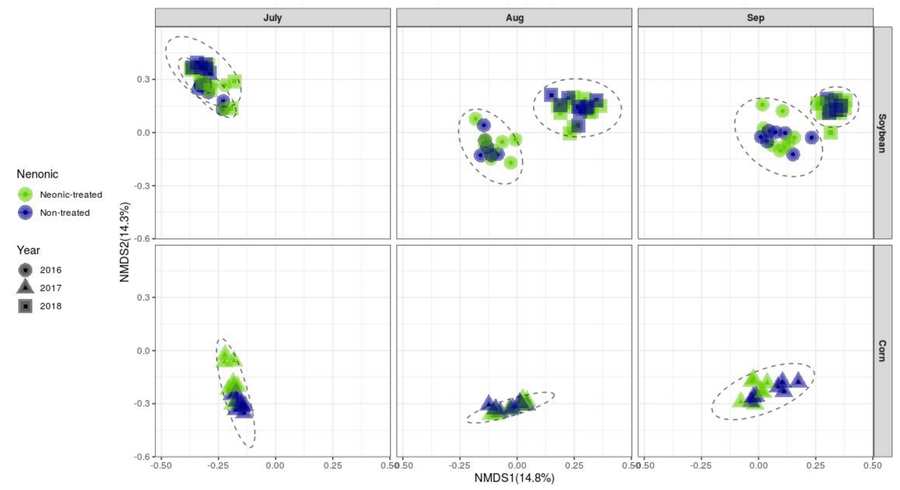
Table 1: Bacterial community taxonomic composition variation of both soybean and corn soil and phyllosphere (blue), as well as soybean (green) and corn (yellow) soil and phyllosphere, separately (PERMANOVA Bray-Curtis dissimilarities).

STUDY DESIGN

Crop	Year	Habitat	Sampling Time: Month	Sampling Time: Stage	Number of Treatments	Replicate	Sample/ Replicate	Total Samples
Soybean	2016 2018	Leaf	July	Full Flowering	2	4	2	48/year
			Aug.	Seeding				
			Sep.	Maturity				
		Soil	May	Planting	2	4	2	64/year
			July	Full Flowering				
			Aug.	Seeding				
			Sep.	Maturity				
Corn	2017	Leaf	July	Tasseling	2	4	2	48
			Aug.	Blister				
			Sep.	Maturity				
		Soil	May	Planting	2	4	2	64
			July	Tasseling				
			Aug.	Blister				
			Sep.	Maturity				

Table 2: Sampling chart.

WANT MORE DETAILS?



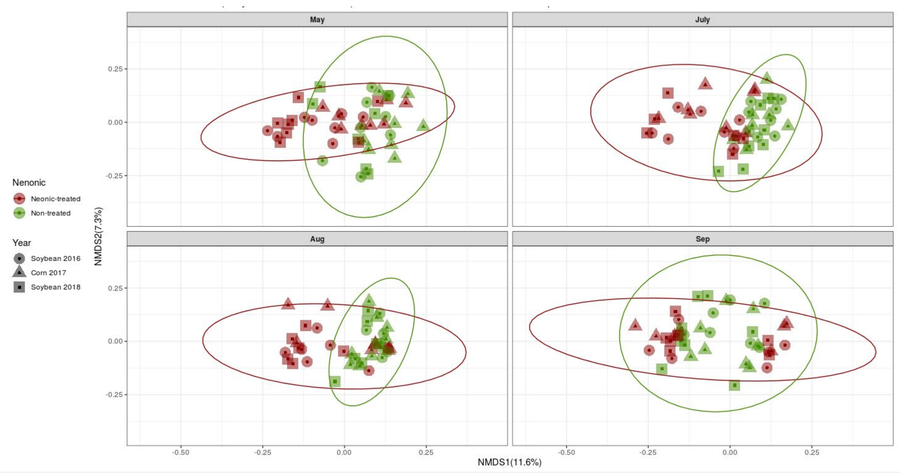


Figure 2: Bray-Curtis based non-metric multidimensional scaling (NMDS) plot of the phyllosphere (top) and soil (bottom) bacterial taxonomic composition show that host species (especially for phyllosphere) and time (month and year) are the main drivers of phyllosphere and soil bacterial diversity and explain it better than neonic treatment. However, the effects of neonic on soil bacterial composition are in interaction with time (especially month). These effects increase in the middle of growing season (July and August) and decrease towards the end of the season. Ellipses represent 99% confidence level.

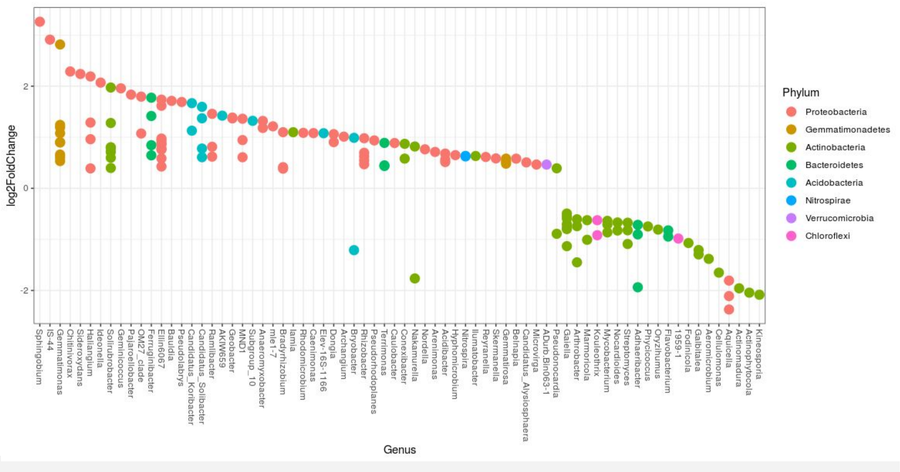


Figure 3: The soybean and corn soil bacterial genera that are significantly associated with neonic treatment. The genera on the top of the graph (log2FoldChange > 0) are related to the non-treated samples, while the others (log2FoldChange < 0) are associated with the neonic-treated samples.