

Microbial community structure changes in three soil types soils exposed to nonsteroidal anti-inflammatory drugs. A laboratory experiment

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Conclusion

Soils are the base of life and they are subjected to multiple anthropogenic pressures

Climate change extreme related events (e.g., extreme precipitation and droughts) are amplifying this impact.

Drought periods and the lack of water are increasing the waste water use in the environment increasing the spread of elements that are not completely removed such as nonsteroidal anti-inflammatory drugs (NSAID's).

Also municipal waste in landfills can increase soil contamination with NSAID's

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NSAID's are the most common used drugs and it is key to understand their behavioral soils

Soils are complex medium and respond differently to the same disturbance, affecting heterogeneously their functions and services

Understanding different microbial response to NSAID's in soil types responses is key to understand their vulnerability/resistance.

This work aims to study the impacts of Ibuprofen, Diclofenac, Mixture (Ibuprofen+ Diclofenac) on different soil types (Cambic chernozem, Luvisols and Calcarius rendzinas)

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Study site

Three different types of soil were sampled (0-30 cm) in Romania

Soil type: Luvisols

Latitude: 46.41 N

Longitude: 23.32 E

Altitude: 717 masl

Land use: Grassland

Soil type: Cambic chernozem

Latitude: 46.47 N

Longitude: 23.39 E

Altitude: 354 masl

Land use: Grassland

Soil type: Calcarius rendzinas

Latitude: 46.44 N

Longitude: 23.33 E

Altitude: 465 masl

Land use: Forest

3 soil types x 5 replicates x 4 treatments Control; Ibuprofen, Diclofenac, Mixture (Ibuprofen+ Diclofenac)x

8 different days (1, 2, 3, 5, 15, 30 and 50 days)

480 samples were processed.

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Cambic chernozem



Luvisol



Calcaric rendzina



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Laboratory procedures

Soils were sieved at <2mm sieve

NSAID's

Amount applied in the soil

Ibuprofen: 1 ug/g

Diclofenac: 1 ug/g

Mixture: 1 ug/g Ibuprofen + 1 ug/g Diclofenac)

NSAID's were assessed using Gas Chromatography-Mass spectrometry, after solid phase micro extraction using 96 well plate polymeric reversed phase cartridge.

More details about the method in **Kovacs et al. (2021)**

Environmental Analysis

Determination of the Uptake of Ibuprofen, Ketoprofen, and Diclofenac by Tomatoes, Radishes, and Lettuce by Gas Chromatography–Mass Spectrometry (GC–MS)

E. D. Kovacs , L. Silaghi-Dumitrescu, M. H. Kovacs & C. Roman

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Soil Microbiom

PLFA, Total bacteria and total fungi abundance and major phenotypic structure were determined by Gas-chromatography and flame ionization detector, using Sherlock MDI microbial identification system
Method details in **Kovacs et al. (2022)**.

Article

Structural and Metabolic Profiling of *Lycopersicon esculentum* Rhizosphere Microbiota Artificially Exposed at Commonly Used Non-Steroidal Anti-Inflammatory Drugs

Emoke Dalma Kovacs ^{1,2,*}, Luminita Silaghi-Dumitrescu ², Cecilia Roman ¹ and Di Tian ³ 

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Statistical analysis

Significant differences between days was identified using non-parametric Friedman ANOVA test

Significant differences between treatments were identified using non-parametric Kruskal-Wallis ANOVA test

Post-hoc test was conducted using a Dunn test

Principal components analysis (Varimax rotated) was conducted to identify the association between the variables

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Total PLFA (nmol/g.dw)

Soil type	Treatment	Day 1	Day 2	Day 3	Day 5	Day 15	Day 30	Day 50	Friedman ANOVA
Cambic Chernozem	Control	91.35 (91.31;92.65) a AB	81.61 (81.52;82.26) a AB	74.19 (73.06;74.23) bc C	103.25 (102.79;103.41) a A	121.05 (121.04;122.42) a A	76.81 (76.32;77.67) a BC	75.42 (72.55;75.82) a BC	***
	Ibuprofen	56.78 (56.66;60.95) a AB	52.39 (52.37;52.44) ab AB	61.66 (60.04;62.09) c AB	64.33 (63.90;65.98) bc A	68.02 (65.07;68.39) c A	27.51 (27.41;27.12) b BC	30.14 (30.05;30.68) ab BC	***
	Diclofenac	52.12 (51.76;52.77) b BC	38.57 (37.36;38.70) b BC	78.37 (77.74;79.91) ab AB	82.85 (82.11;84.43) ab A	81.65 (81.50;81.72) bc AB	34.02 (33.77;34.12) ab C	24.29 (24.23;24.45) bc C	***
	Mixture	55.26 (55.21;55.82) ab AB	38.61 (38.34;38.77) b AB	87.89 (85.82;88.97) a A	43.32 (42.35;44.65) c AB	89.94 (89.54;89.96) ab A	25.80 (25.57;26.78) b B	22.69 (22.60;23.28) c B	***
Kruskal-Wallis	p-value	**	***	***	***	***	***	***	

Table 1. Median (1st quartile - 3rd quartile) amount of Total PLFA in Control, Ibuprofen, Diclofenac and mixture (Ibuprofen+ Diclofenac) treated Cambic Chernozem. **p<= 0.01 and ***- p<= 0.001

Total PLFA (nmol/g.dw)

Soil type	Treatment	Day 1	Day 2	Day 3	Day 5	Day 15	Day 30	Day 50	Friedman ANOVA
Luvisoil	Control	61.43 (60.10;61.64) a A	60.90 (58.46;61.73) a A	59.32 (59.30;59.73) b A	57.04 (56.83;57.88) a AB	56.68 (56.07;57.02) a AB	51.01 (50.20;52.44) a B	51.07 (50.54;52.06) a B	***
	Ibuprofen	30.45 (30.29;31.64) b ABC	22.85 (22.28;23.13) b C	67.72 (66.80;68.97) ab A	40.55 (40.40;41.24) b AB	44.07 (43.94;44.30) ab AB	25.65 (25.14;26.39) b BC	21.57 (20.70;21.92) c C	***
	Diclofenac	28.66 (28.17;28.71) bc BC	16.84 (16.68;17.07) c C	69.65 (68.14;71.62) ab A	43.61 (43.31;44.07) ab AB	34.98 (34.95;35.36) b AB	26.26 (26.17;27.28) b B	53.57 (52.64;53.78) a AB	***
	Mixture	26.46 (26.31;26.64) c BC	18.62 (18.62;18.96) bc C	73.74 (73.50;74.09) a A	33.58 (33.46;33.70) c AB	54.73 (54.64;56.47) a AB	44.88 (44.63;45.01) ab AB	27.13 (24.71;27.92) ab BC	***
Kruskal-Wallis	p-value	***	***	**	***	***	***	***	

Table 2. Median (1st quartile - 3rd quartile) amount of Total PLFA in Control, Ibuprofen, Diclofenac and mixture (Ibuprofen+ Diclofenac) treated Luvisoil. **p<= 0.01 and ***- p<= 0.001

Total PLFA (nmol/g.dw)

Soil type	Treatment	Day 1	Day 2	Day 3	Day 5	Day 15	Day 30	Day 50	Friedman ANOVA
Calcareous rendzinas	Control	72.51 (71.94;74.36) ab A	62.46 (61.46;62.48) a B	61.10 (59.41;62.56) ab B	68.99 (68.95;69.22) a AB	74.54 (74.21;74.88) a A	64.61 (63.59;65.37) a B	66.23 (66.19;67.19) a B	***
	Ibuprofen	46.67 (45.98;46.69) c A	57.23 (56.61;57.58) ab A	39.83 (38.94;40.32) b AB	34.35 (33.45;36.38) bc ABC	38.54 (38.43;38.93) c BC	25.80 (25.64;25.85) ab BC	23.47 (22.92;24.08) b C	***
	Diclofenac	74.57 (74.25;74.98) a A	35.09 (34.86;36.09) c AB	74.47 (74.03;76.17) a A	28.89 (27.00;29.58) c AB	49.00 (49.32;49.09) bc A	19.67 (19.54;22.07) c C	25.04 (25.02;25.56) ab BC	***
	Mixture	62.26 (62.25;62.81) bc A	38.40 (38.20;38.67) bc ABC	64.48 (63.92;64.65) ab A	41.83 (41.35;41.91) ab ABC	60.29 (60.28;60.78) ab AB	23.36 (23.35;23.72) bc BC	22.86 (22.79;23.03) b C	***
Kruskal-Wallis	p-value	***	***	***	***	***	***	**	

Table 3. Median (1st quartile - 3rd quartile) amount of Total PLFA in Control, Ibuprofen, Diclofenac and mixture (Ibuprofen+ Diclofenac) treated Calcareous rendzinas. **p<= 0.01 and ***- p<= 0.001

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Total Bacteria (nmol/g.dw)

Soil type	Treatment	Day 1	Day 2	Day 3	Day 5	Day 15	Day 30	Day 50	Friedman ANOVA
Cambic Chernozem	Control	80.15 (79.41;80.50) a AB	74.05 (73.90;74.61) a AB	65.32 (65.15;65.46) bc C	91.04 (91.04;91.06) a A	108.08 (107.22;108.63) a A	71.76 (71.46;72.05) a BC	72.49 (69.94;73.65) a BC	***
	Ibuprofen	51.33 (51.05;52.39) ab AB	47.68 (47.66;47.94) ab AB	54.08 (53.81;54.58) c AA	57.79 (57.76;58.28) bc AB	62.45 (59.64;62.68) c A	26.09 (25.86;26.70) bc BC	24.72 (24.39;25.10) ab C	***
	Diclofenac	46.84 (46.52;47.73) b AB	35.04 (34.29;35.44) b B	70.00 (69.09;70.07) ab A	73.32 (72.54;73.74) ab A	74.99 (74.20;75.42) bc A	32.36 (32.31;32.65) ab B	23.66 (23.57;23.85) ab B	***
	Mixture	49.97 (49.77;50.27) b BC	35.12 (35.08;35.25) b BC	75.34 (75.24;75.86) a AB	39.40 (38.43;40.46) c BC	79.89 (79.71;80.14) ab A	24.87 (24.59;25.11) c CD	21.86 (21.74;22.75) b D	***
Kruskal-Wallis	p-value	**	**	***	***	***	***	***	

Table 4. Median (1st quartile - 3rd quartile) amount of Total Bacteria in Control, Ibuprofen, Diclofenac and mixture (Ibuprofen+ Diclofenac) treated Cambic Chernozem. **p<= 0.01 and ***- p<= 0.001

Total Bacteria (nmol/g.dw)

Soil type	Treatment	Day 1	Day 2	Day 3	Day 5	Day 15	Day 30	Day 50	Friedman ANOVA
Luvosoil	Control	52.67 (52.56;53.82) a A	54.93 (52.22;56.81) a A	51.47 (51.46;51.58) b AB	51.48 (50.73;51.85) a AB	50.41 (49.39;50.73) a AB	47.10 (45.89;48.71) a B	46.95 (46.88;48.30) a B	***
	Ibuprofen	27.32 (27.30;27.60) ab AB	20.75 (20.64;21.21) ab B	59.56 (59.42;60.88) ab A	37.34 (37.12;37.76) bc AB	41.60 (41.52;41.73) ab A	24.42 (24.35;25.21) b B	20.84 (20.29;21.01) b B	***
	Diclofenac	25.25 (25.06;25.26) bc B	16.23 (16.08;16.37) c B	61.05 (60.72;62.76) ab A	40.14 (39.92;41.26) ab AB	33.64 (33.39;34.01) b AB	25.45 (24.79;25.59) b B	47.74 (47.59;49.03) a A	***
	Mixture	23.20 (23.13;23.23) c BC	17.82 (17.82;18.17) bc C	64.22 (64.20;65.12) a A	31.65 (30.66;31.87) c ABC	50.90 (50.72;52.04) a A	41.61 (41.42;42.01) ab AB	25.08 (22.22;25.66) ab BC	***
Kruskal-Wallis	p-value	***	***	***	***	***	***	**	

Table 5. Median (1st quartile - 3rd quartile) amount of Total Bacteria in Control, Ibuprofen, Diclofenac and mixture (Ibuprofen+ Diclofenac) treated Luvisol. **p<= 0.01 and ***- p<= 0.001

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Total Bacteria (nmol/g.dw)

Soil type	Treatment	Day 1	Day 2	Day 3	Day 5	Day 15	Day 30	Day 50	Friedman ANOVA
Calcareous rendzinas	Control	65.72 (64.87;66.92) a AB	55.05 (54.80;55.81) a C	55.82 (54.40;56.86) bc C	62.66 (62.54;62.84) a ABC	68.95 (68.70;69.22) a A	58.97 (58.53;60.02) a AB	66.23 (66.19;66.61) a AB	***
	Ibuprofen	41.90 (41.85;42.02) b AB	51.73 (51.60;52.16) ab A	36.62 (36.32;37.62) c AB	33.00 (32.01;35.08) bc BC	37.56 (37.38;37.76) c AB	24.61 (24.56;24.81) ab BC	23.18 (22.60;23.63) b BC	***
	Diclofenac	66.85 (66.59;66.98) a A	33.21 (31.89;33.67) c ABC	66.00 (65.61;66.47) a A	28.41 (26.52;29.11) c BC	47.14 (46.69;47.18) bc	18.74 (18.42;21.31) c C	24.20 (24.18;24.85) ab BC	***
	Mixture	54.67 (54.36;54.79) ab A	35.35 (35.06;35.60) bc BC	58.88 (58.72;58.93) ab A	39.92 (39.60;40.00) ab ABC	56.23 (56.07;56.31) ab A	23.12 (23.09;23.37) bc C	22.42 (22.33;22.54) b C	***
Kruskal-Wallis	p-value	***	***	***	***	***	***	**	

Table 6. Median (1st quartile - 3rd quartile) amount of Total Bacteria in Control, Ibuprofen, Diclofenac and mixture (Ibuprofen+ Diclofenac) treated Calcareous rendzinas. **p<= 0.01 and ***- p<= 0.001

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Total Fungi (nmol/g.dw)

Soil type	Treatment	Day 1	Day 2	Day 3	Day 5	Day 15	Day 30	Day 50	Friedman ANOVA
Cambic Chernozem	Control	8.64 (8.41;8.65) a B	5.13 (4.97;5.17) a BC	5.64 (5.45;5.67) ab BC	9.79 (9.77;9.88) a AB	10.80 (10.35;10.82) a A	4.76 (4.66;5.00) a CD	1.54 (1.26;1.54) a D	***
	Ibuprofen	4.69 (4.44;4.76) ab A	4.05 (3.94;4.08) ab A	4.48 (4.23;4.58) b A	5.10 (4.51;5.22) bc A	3.87 (3.71;3.89) c A	0.71 (0.75;0.71) b B	1.59 (1.27;1.60) a B	***
	Diclofenac	4.24 (4.19;4.28) b BC	2.65 (2.45;2.66) b BC	6.38 (6.26;6.52) a AB	7.12 (6.91;7.58) ab A	5.83 (5.60;5.86) bc AB	1.18 (1.14;1.19) ab C	0.32 (0.31;0.39) ab C	***
	Mixture	4.16 (4.12;4.27) b B	2.66 (2.66;2.87) b BC	8.28 (8.11;8.67) a A	2.41 (2.31;2.52) c BC	6.81 (6.65;7.23) ab AB	1.09 (0.56;1.13) b BC	0.17 (0.17;0.18) b C	***
Kruskal-Wallis	p-value	**	***	***	***	***	**	**	

Table 7. Median (1st quartile - 3rd quartile) amount of Total Fungi in Control, Ibuprofen, Diclofenac and mixture (Ibuprofen+ Diclofenac) treated Cambic chernozem. **p<= 0.01 and ***- p<= 0.001

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Total Fungi (nmol/g.dw)

Soil type	Treatment	Day 1	Day 2	Day 3	Day 5	Day 15	Day 30	Day 50	Friedman ANOVA
Luvosoil	Control	5.23 (5.02;5.30) a A	4.87 (4.85;4.92) a AB	5.02 (4.99;5.10) b A	4.55 (4.41;4.60) a AB	5.01 (4.82;5.02) a AB	2.47 (2.46;2.69) a B	2.55 (2.34;2.76) ab B	***
	Ibuprofen	1.66 (1.65;1.90) ab AB	1.10 (1.08;1.12) ab C	5.83 (5.33;5.89) ab A	1.75 (1.75;1.79) b ABC	1.54 (1.33;1.65) b C	0.71 (0.71;0.73) b C	0.50 (0.41;0.50) b C	***
	Diclofenac	1.62 (1.42;1.63) b BC	0.61 (0.60;0.61) c D	5.57 (5.32;5.65) ab A	2.00 (1.92;2.07) ab BC	1.35 (1.31;1.35) b BC	0.72 (0.72;0.72) b CD	3.44 (3.41;3.68) a AB	***
	Mixture	1.53 (1.49;1.53) b ABC	0.80 (0.80;0.80) bc C	6.20 (6.19;6.32) a A	1.38 (1.37;1.68) b ABC	3.15 (3.15;3.18) ab AB	2.34 (2.27;2.36) ab AB	0.54 (0.46;0.55) b C	***
Kruskal-Wallis	p-value	**	***	**	**	***	**	**	

Table 8. Median (1st quartile - 3rd quartile) amount of Total Fungi in Control, Ibuprofen, Diclofenac and mixture (Ibuprofen+ Diclofenac) treated Luvisoil. **p<= 0.01 and ***- p<= 0.001

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Total Fungi (nmol/g.dw)

Soil type	Treatment	Day 1	Day 2	Day 3	Day 5	Day 15	Day 30	Day 50	Friedman ANOVA
Calcareous rendzinas	Control	5.73 (5.67;5.88) a A	4.67 (4.64;4.90) a AB	3.84 (3.79;3.92) ab BC	5.35 (5.21;5.37) a AB	4.68 (4.63;4.74) a ABC	4.36 (4.35;4.55) a BC	0.00 (0;0) - C	***
	Ibuprofen	3.76 (3.46;3.76) b A	4.65 (4.60;4.67) a A	2.00 (1.96;2.05) b AB	1.30 (1.25;1.35) bc B	0.95 (0.95;0.96) c BC	0.41 (0.39;0.43) ab C	0.00 (0;0) - C	***
	Diclofenac	6.10 (6.08;6.11) a A	2.14 (1.90;2.16) b AB	5.17 (4.97;5.34) a A	0.48 (0.47;0.48) c B	1.53 (1.49;1.72) bc B	0.38 (0.37;0.41) ab B	0.00 (0;0.11) - B	***
	Mixture	5.44 (5.44;5.59) ab A	2.83 (2.83;2.85) ab AB	3.82 (3.80;3.96) ab A	1.73 (1.73;1.75) ab BC	3.34 (3.34;3.37) ab ABC	0.28 (0.23;0.34) c C	0.14 (0.12;0.15) - C	***
Kruskal-Wallis	p-value	**	***	**	***	***	**	NS	

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Total PLFA PCA

Cambic Chernozem (CC)

Luvisols (L)

Calcareous rendzinas (CR)

□ Day 1

◇ Day 2

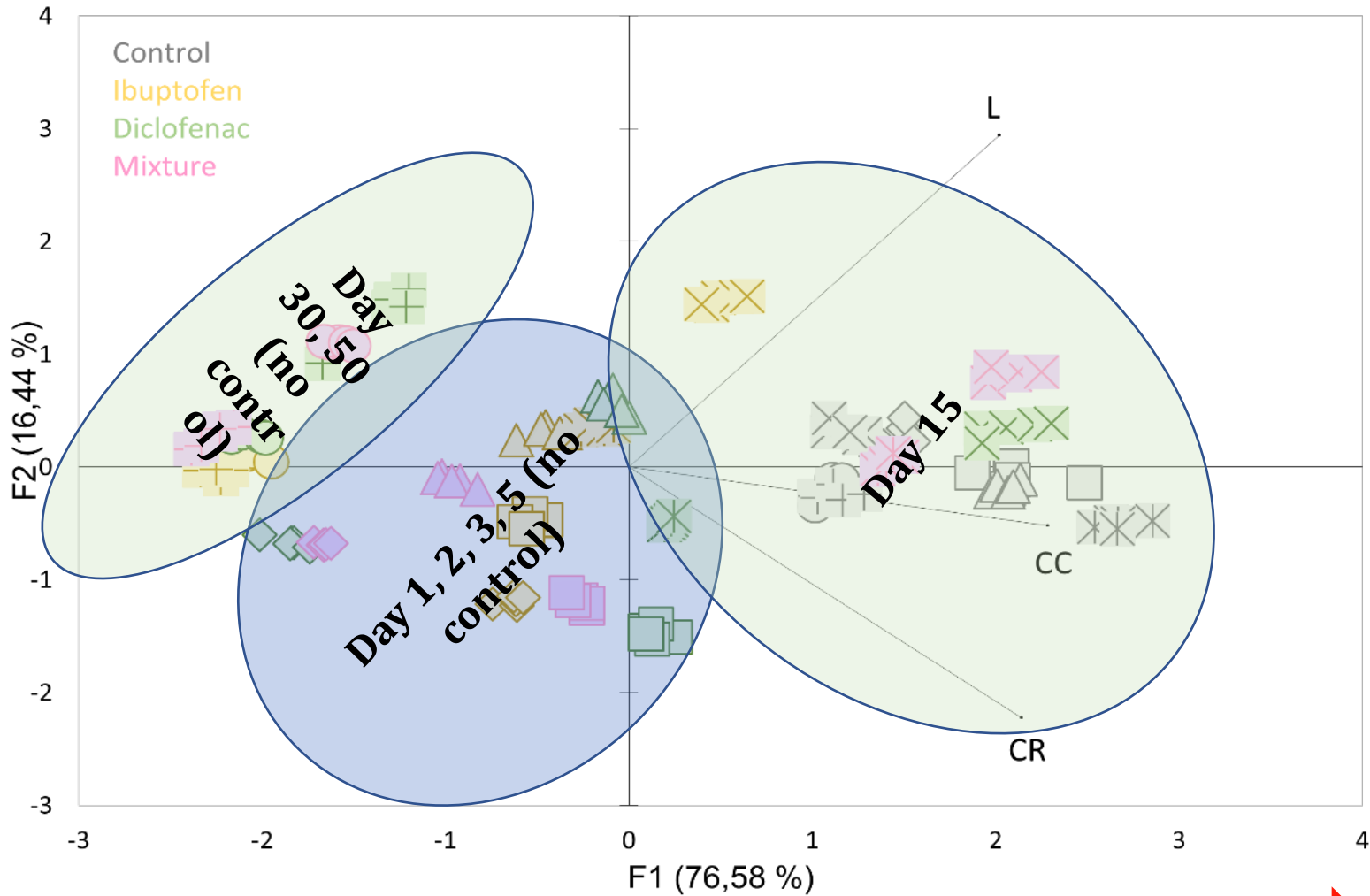
⊠ Day 3

△ Day 5

⊞ Day 15

○ Day 30

⊞ Day 50



Total Bacteria PCA

Cambic Chernozem (CC)

Luvisols (L)

Calcareous rendzinas (CR)

□ Day 1

◇ Day 2

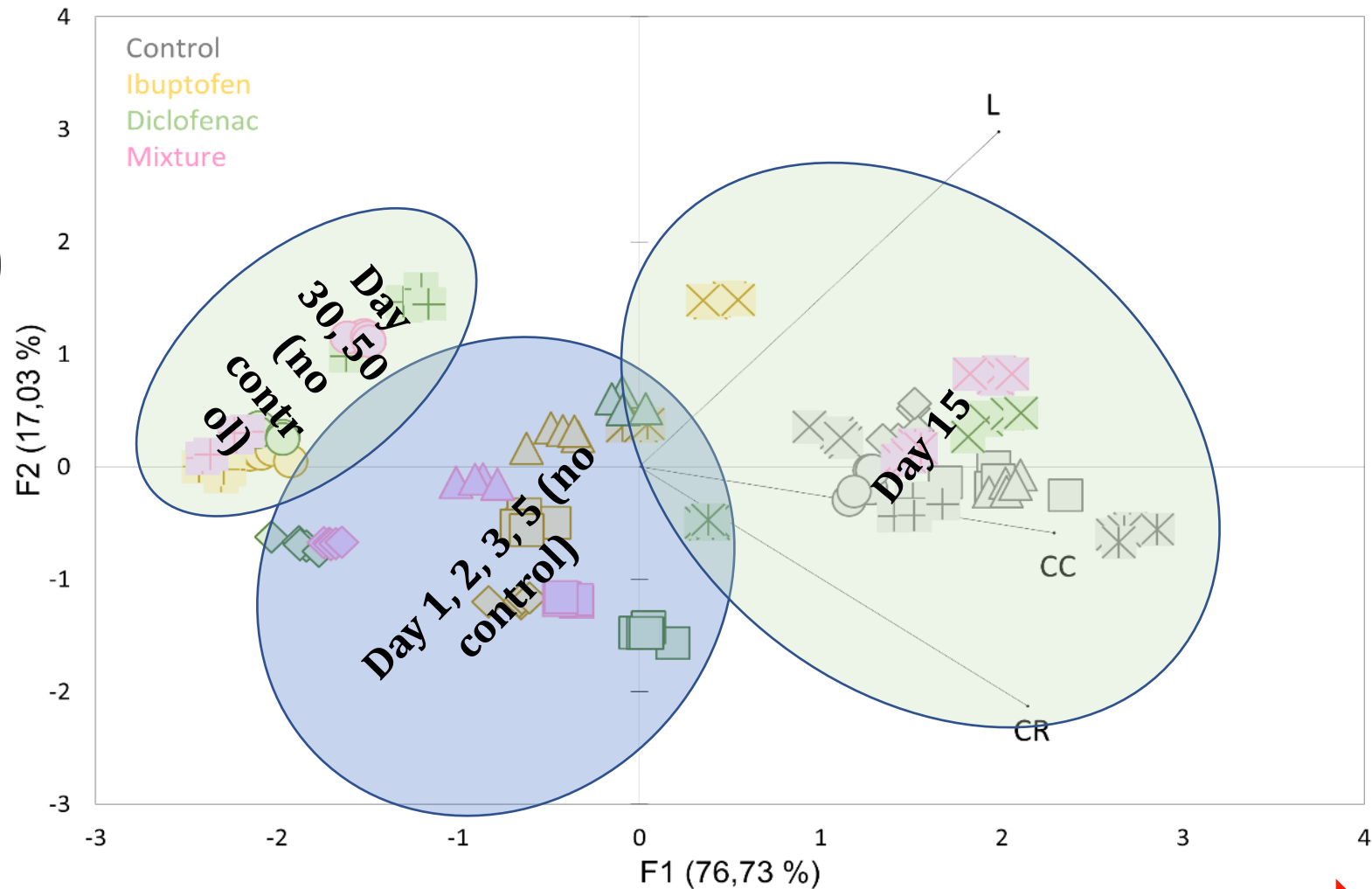
⊠ Day 3

△ Day 5

⊠ Day 15

○ Day 30

⊠ Day 50



Total Fungi PCA

Cambic Chernozem (CC)

Luvisols (L)

Calcareous rendzinas (CR)

□ Day 1

◇ Day 2

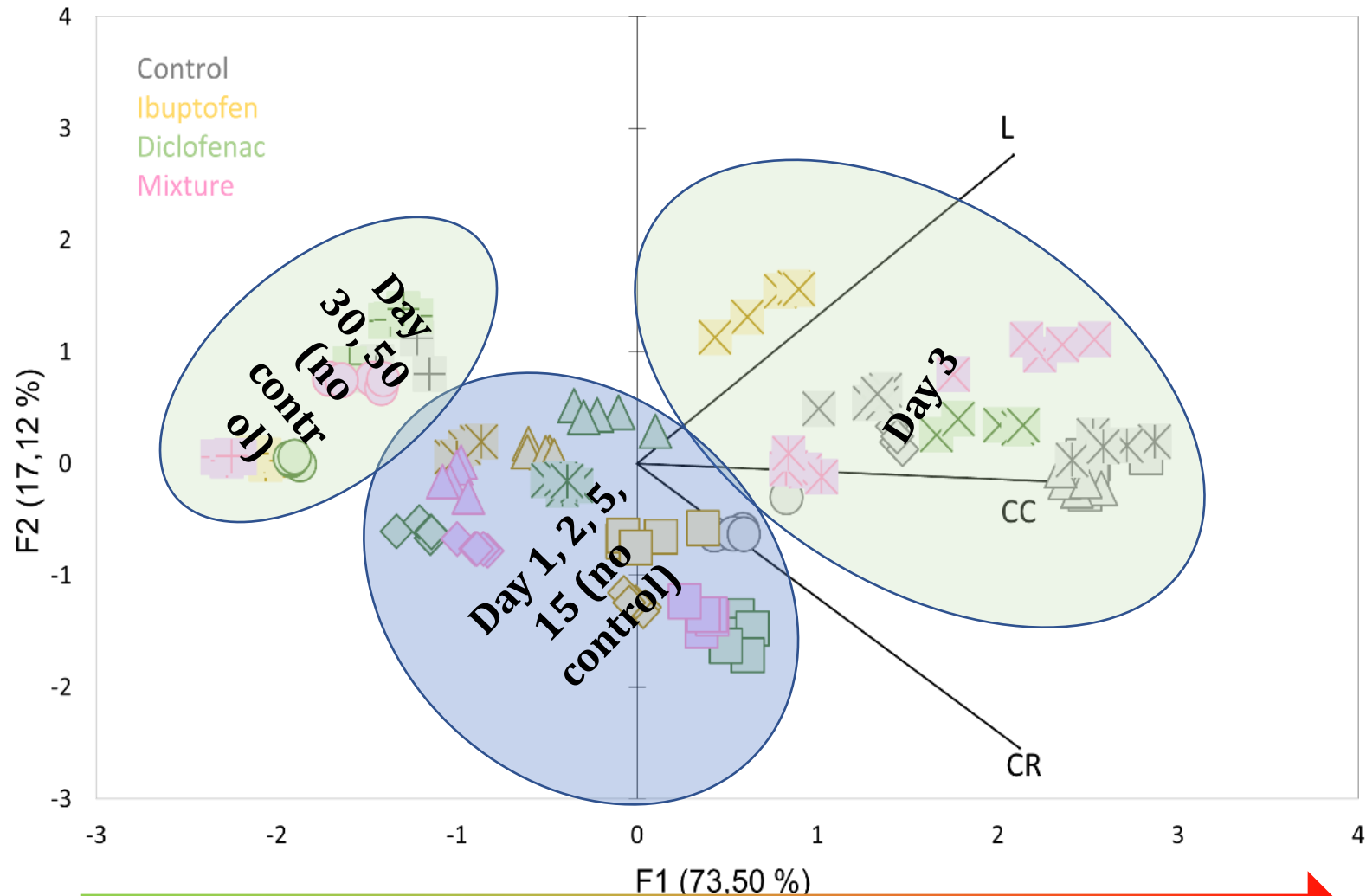
⊠ Day 3

△ Day 5

⊞ Day 15

○ Day 30

⊞ Day 50



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Cambic chernozem

PLFA

Highest value time: 15 days

Highest value treatment: Control (except for day 3:mixture)

Total bacteria

Highest value time: 15 days

Highest value treatment: Control (except for day 3:mixture)

Total fungi

Highest value time: 3 days (mixture)/ 5 days (Ibuprofen and Diclofenac)

Highest value treatment: Control (except for day 3:mixture)

PLFA and Total bacteria have a same behavior to NSAID's exposure.
Total fungi: respond differently to NSAID's type. Respond faster to mixture

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Luvisol

PLFA

Highest value time: 3 days

Highest value treatment: Control (except for day 3:mixture)

Total bacteria

Highest value time: 3 days

Highest value treatment: Control (except for day 3:mixture)

Total fungi

Highest value time: 3 days

Highest value treatment: Control (except for day 3:mixture)

PLFA, Total bacteria and Total fungi have a same behavior to NSAID's exposure.

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Calcareous rendzinas

PLFA

Highest value time: 3 days

Highest value treatment: Control (except day 1 and day 3: Diclofenac)

Total bacteria

Highest value time: 15 days (Ibuprofen) 3 days (Diclofenac and mixture)

Highest value treatment: Control (except for day 3: mixture)

Total fungi

Highest value time: 1 day (Diclofenac and Mixture), 2 days (Ibuprofen)

Highest value treatment: Control (except for day 3: mixture)

PLFA, Total bacteria and Total fungi have a different behavior to NSAID's exposure. Microbiological response is different according to the NSAID.

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Generally NSAID's have a negative impact on microbiological activity and changed soil functions and likely services

PLFA, Total bacteria and Total fungi in the different soils respond heterogeneously to NSAID's exposure

Calcareous rendzinas microbiom are less resistant and have the lowest resistant to NSAID's exposure. Cambic Chernozem microbiom appear to have a highest resistance. Luvisols microbiom are in an intermediate position

It is important to understand what's happen in the 3 day after exposure in mixture contamination.

The increase of microbiome abundance in the different days of exposure was likely due to the capacity of microbes to their capacity to degrade NSAID's or use it as resources (e.g., carbon)

30 and 50 days after exposure the treatment PLFA, Total bacteria and total fungi drop likely due negative impacts on NSAID's.

Thank you very much for the attention

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