

# **Microbe-Mediated Organic Fertilization Increases Insect Predator Attraction Upon Fruit Damage in Olive Trees**

**Running Title: Soil Organic Fertilization Boosts Olive Tree-Induced Defense**

Martin Aguirrebengoa<sup>a</sup>, Beatriz Moreno<sup>a</sup>, Nuria Guirado<sup>a</sup>, Rafael Núñez<sup>b</sup>, María L. Fernández-Sierra<sup>a</sup>, Fernando Reyes<sup>c</sup>, Jesús Martín<sup>c</sup>, Emilio Benítez<sup>a\*</sup>

<sup>a</sup>Department of Biotechnology and Environmental Protection, Estación Experimental del Zaidín (EEZ), CSIC, c/ Profesor Albareda 1, 18008 Granada, Spain

<sup>b</sup> Scientific Instrumentation Service, Estación Experimental del Zaidín (EEZ), CSIC, c/ Profesor Albareda 1, 18008 Granada, Spain

<sup>c</sup> Fundación MEDINA, Avda. del Conocimiento 34, 18016, Armilla, Granada, Spain

\*Corresponding author: [emilio.benitez@eez.csic.es](mailto:emilio.benitez@eez.csic.es)

**Supplementary Table S1.** Identified bacterial enzymes and fungal lifestyles.

Identified bacterial enzymes (BacDive)		
4-nitrophenyl- $\beta$ -D-galactopyranoside	DNase	lysine decarboxylase
glutamate dehydrogenase	phosphatase	cystine arylamidase
endo-1,4 $\beta$ -xylanase	6-phospho $\beta$ -galactosidase	ornithine decarboxylase
$\beta$ -maltosidase	leucine aminopeptidase	trypsin pyrazinamidase
lipase Tween 80	nitrate reductase	pyrazinamidase
tryptophan decarboxylase	chymotrypsin	pyrrolidonyl arylamidase
$\alpha$ -amylase	$\alpha$ -arabinosidase	alanyl-phenylalanyl-proline arylamidase
glutamyl arylamidase pNA	esterase	valine arylamidase
chitinase	$\alpha$ -maltosidase	arginine dihydrolase
$\beta$ -xylosidase	L-aspartate arylamidase	N-acetyl- $\beta$ -glucosaminidase
arginine decarboxylase	cytochrome-c oxidase	gelatinase
phosphohydrolase	gamma-glutamyltransferase	$\alpha$ -galactosidase
lecithinase	tryptophan deaminase	urease
2-nitrophenyl $\beta$ -D-galactopyranoside	$\alpha$ -fucosidase	naphthol-AS-BI-phosphohydrolase
caseinase	lipase	esterase C 4
$\beta$ -galactopyranosidase	serine arylamidase	esterase lipase C 8
cellulase	lipase C 14	$\alpha$ -glucosidase
tripeptide aminopeptidase	$\alpha$ -mannosidase	acid phosphatase
phosphoamidase	phenylalanine arylamidase	cytochrome oxidase
phenylalanine deaminase	tyrosine arylamidase	$\beta$ -glucosidase
amylase	glycyl tryptophan arylamidase	leucine arylamidase
$\beta$ -D-fucosidase	proline arylamidase	$\beta$ -galactosidase
arylsulfatase	$\alpha$ -chymotrypsin	catalase
protease	alanine arylamidase	alkaline phosphatase
valine aminopeptidase	$\beta$ -glucuronidase	
Identified fungal lifestyles (FungalTraits)		
animal decomposer	hypervariable	root pathogen
arbuscular mycorrhizal	litter saprotroph	soil saprotroph
dung saprotroph	plant aerial tissue pathogen	unspecified saprotroph
ectomycorrhizal	root associated	unspecified symbiotroph
ericoid mycorrhizal	root endophyte	wood saprotroph
foliar endophyte		

**Supplementary Table S2.** Relative areas of components detected in the LC/HRMS analysis of *Prays oleae* larva (L) and the product of its digestion (D) extracts; A: non-attacked seeds control.

Compound/molecular formula	Exact Mass of adduct used for relative quantification	Adduct identity	A	L	D
$C_9H_{12}O_3$	169.086±0.005	M+H <sup>+</sup>	200990	81928	7562642
$C_{10}H_{16}O_5$	185.081±0.005	M-MeOH+H <sup>+</sup>	39052	170444	1225207
$C_7H_8N_4O_2$	219.028±0.005	M+K <sup>+</sup>	738310	49183	1092571
$C_{11}H_{16}O_5$	229.107±0.005	M+H <sup>+</sup>	571550	266421	2619806
Lisianthioside	734.287±0.005	M+NH <sub>4</sub> <sup>+</sup>	238501	475	565759
$C_{31}H_{42}O_{17}$	704.276±0.005	M+NH <sub>4</sub> <sup>+</sup>	784556	916	2347131
$C_{34}H_{46}O_{21}$	808.287±0.005	M+NH <sub>4</sub> <sup>+</sup>	175548	12311	288071
$C_{34}H_{44}O_{20}$	790.276±0.005	M+NH <sub>4</sub> <sup>+</sup>	260352	675	301374
$C_{48}H_{64}O_{27}$	1090.397±0.005	M+NH <sub>4</sub> <sup>+</sup>	169138	1212	398005
Oleopolynuzhenide A	747.276±0.005	M+2NH <sub>4</sub> <sup>+</sup>	494202	13697	1811372
Jaspolyoleoside B/C	666.249±0.005	M+2NH <sub>4</sub> <sup>+</sup>	1728972	0	810683

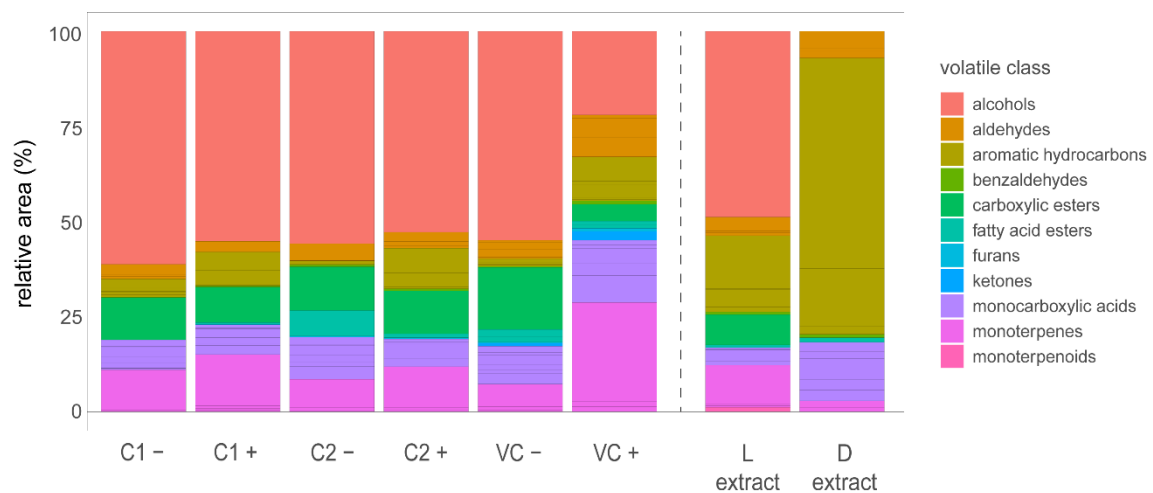
**Supplementary Table S3.** PERMANOVA results of soil and simulated fruit damage treatments and their interaction on taxonomic and functional beta-diversity of bacteria and fungi.

	<b>Soil treatment</b>		<b>Simulated fruit damage</b>		<b>Soil x Simulated fruit damage</b>		<b>R<sup>2</sup></b>
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	
taxonomic diversity bacteria	1.02	0.37	0.85	0.52	0.69	0.85	0.077
functional diversity bacteria	0.83	0.45	0.37	0.58	0.74	0.48	0.064
taxonomic diversity fungi	1.49	0.075	0.90	0.50	1.51	0.066	0.119
functional diversity fungi	1.23	0.27	1.10	0.33	1.46	0.19	0.111

**Supplementary Table S4.** Results of linear mixed models for the effects of best subsets of volatiles explaining the mean preference of *C. carnea* in each C1- vs. C1+, C2- vs. C2+, C1+ vs. C2+ and C2+ vs. VC+ comparison.

Comparison – best volatile subsets	<i>F</i>	<i>P</i>	R <sup>2</sup> <sub>m</sub>
<b>C1- vs C1+</b> ethylbenzene + furan, 2-pentyl + hexanoic acid + nonanal + nonanoic acid + octanoic acid	6.37	0.041	0.500
<b>C2- vs C2+</b> ethyl alcohol-isopropyl alcohol + furan, 2-pentyl + hexanoic acid, methyl ester + nonanoic acid + octanoic acid acetic acid + pentanoic acid	12.21	0.017	0.622
<b>C1+ vs C2+</b> 5-hepten-2-one, 6-methyl + ethyl alcohol-isopropyl alcohol + ethylbenzene + hexadecanoic acid, methyl ester + hexanoic acid, methyl ester + octanal	25.35	0.009	0.750
5-hepten-2-one, 6-methyl + ethyl alcohol-isopropyl alcohol + ethylbenzene + hexadecanoic acid, methyl ester + hexanoic acid, methyl ester + pentanoic acid	25.35	0.009	0.750
5-hepten-2-one, 6-methyl + ethyl alcohol-isopropyl alcohol + ethylbenzene + hexanoic acid, methyl ester + octanal + pentanoic acid	25.35	0.009	0.750
5-hepten-2-one, 6-methyl + ethyl alcohol-isopropyl alcohol + hexadecanoic acid, methyl ester + hexanoic acid, methyl ester + octanal + pentanoic acid	25.35	0.009	0.750
ethyl alcohol-isopropyl alcohol + ethylbenzene + hexadecanoic acid, methyl ester + hexanoic acid, methyl ester + octanal + pentanoic acid	25.35	0.009	0.750
<b>C2+ vs VC+</b> ethyl alcohol-isopropyl alcohol + ethylbenzene + formic acid, 1- methylethyl ester + nonanal + nonanoic acid	2.69	0.13	0.32

**Supplementary Figure S1.** Mean relative emission area of each volatile compound in experimental treatment plants and extract plants, grouped by volatile class.





**Supplementary Figure S3.** Pairwise Spearman rank correlation coefficients between the 28 volatile organic compounds in experimental plants. The color of the circle indicates the sign of the correlation (red = negative, blue = positive), while the size of the circle indicates the magnitude of the correlation.

