

1 **The use of directional traps for the assessment of short-term phenanthrene**
2 **effects upon soil springtail communities**

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11

12 **Abstract**

13

14 Directional traps, both horizontal and vertical, were used to assess the
15 behavioural impact of phenanthrene application on soil springtail communities.
16 Avoidance was not detected. Rather, a vertical attraction of the dominant species,
17 *Folsomia manolachei*, was demonstrated, as well as a decrease in horizontal
18 movements of *Lepidocyrtus lanuginosus*, another important species mainly captured at
19 the soil surface. Ecological consequences of the results are discussed.

20

21 *Keywords:* Phenanthrene; Springtails; Directional traps; Behaviour

22

23 **1. Introduction**

24

25 The assessment of environmental hazards is still mostly achieved through
26 laboratory tests using species which have been selected for the ease to obtain them in
27 high numbers (Dorn et al., 1998; Fountain and Hopkin, 2005). There is a need for field-
28 relevant methods which can be used to predict changes that may occur at the

1 community level under the influence of a contaminant (Fent, 2003; Fountain and
2 Hopkin, 2004b). Field sampling along transects of increasing contamination has been
3 used for studying the impact of environmental hazards on soil animal communities
4 (Bengtsson and Rundgren, 1988; Erstfeld and Snow-Ashbrook, 1999; Fountain and
5 Hopkin, 2004a). Ecotoxicological tests and field monitoring of populations are reliable
6 methods for the assessment of contamination end-points, but they do not inform us
7 about the mechanisms in play and the way by which populations become
8 depauperated.

9

10 The toxicity and the bio-accumulation of pollutants have been well studied in
11 soil animals (Hopkin et al., 1989; Crouau et al., 1999; Johnson et al., 2002), but still
12 little is known about behavioural aspects, in particular the effects of pollutants on the
13 movement of animals in terrestrial ecosystems (Yearley et al., 1996; Michelozzi et al.,
14 1997; da Luz et al., 2004). It has been shown that some springtail species are able to
15 avoid contaminants while others, belonging to the same community, did not or even
16 were attracted to them (Tranvik and Eijsackers, 1989; Filser et al., 2000; Chauvat and
17 Ponge, 2002). Avoidance movement can be used by an animal to escape a pollutant,
18 provided it can reach more or less rapidly a refuge (Shakir Hanna and Weaver, 2002),
19 but attraction or paralysis might on the contrary entrap it in a toxic environment
20 (Eijsackers, 1978; Fábíán and Petersen, 1994; Petersen and Gjelstrup, 1998). The
21 choice of food is another important clue and, if as a general rule animals avoid toxic
22 food (Visser and Whittaker, 1977; Filser and Hölscher, 1997; Michelozzi et al., 1997),
23 definite attraction to harmful food items may sometimes be observed (Sadaka-Laulan
24 et al., 1998).

25

26 Directional traps have been used to follow horizontal movements of surface
27 animals in patchy environments (Duelli et al., 1990). We suggest that they can be also
28 used to follow horizontal as well as vertical movements of soil animal populations in the

1 presence of contaminants, in particular hydrocarbons, the smell of which may act as a
2 behavioural signal, as this has been demonstrated with fungal odours (Bengtsson et
3 al., 1988, 1991; Hedlund et al., 1995). Avoidance of crude petroleum by earthworms
4 has been observed (Shakir Hanna and Weaver, 2002), but what happens in the case of
5 complex assemblages such as soil springtail communities? In an experimental study of
6 the effects of naphthalene application on the ground, Best et al. (1978) concluded that
7 the horizontal movement of arthropods was negatively affected by naphthalene and
8 that vertical movement was probable, although their sampling methods were
9 inappropriate to fully investigate the latter phenomenon. We thought that the
10 incorporation of horizontal and vertical directional traps in the soil could allow to
11 demonstrate the existence or inexistence of movements of invertebrate populations
12 when hydrocarbons are deposited at the ground surface. Phenanthrene was used,
13 being one of the main components of crude oil as well as of numerous industrial waste
14 products (Bouchez et al., 1996). It is also known for its toxicity and mutagenicity to a
15 wide range of organisms (Schirmer et al., 1998; Crouau et al., 1999; Landrum et al.,
16 2003).

17

18 **2. Material and methods**

19

20 Phenanthrene, $C_{14}H_{10}$, a weakly-soluble 3-cycle PAH (1.6 mg.L^{-1} at 15°C in
21 water), was used in solid state, in the form of a fine white powder (purity 98%) with
22 moderate smell, purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

23

24 The soil used for the experiment stemmed from a woodlot mainly comprised of
25 oak [*Quercus petraea* (Mattus.) Liebl.] and hornbeam (*Carpinus betulus* L.) at the
26 inside of the Park of the Laboratory (Brunoy, France, 20 km South-East of Paris). It
27 was a black rendzina rich in clay (28.2%) and organic carbon (4.7%), with a near
28 neutral $\text{pH}_{\text{H}_2\text{O}}$ (7.4) and a low C/N (11.8) in the top 10 cm (Vannier, 1970). The humus

1 form was an earthworm mull, called calcic Eumull according to Brêthes et al. (1995).
2 Detailed vegetation, soil and climate data can be found in Vannier (1970). Collembolan
3 communities living in the topsoil were described in Chauvat and Ponge (2002) and
4 Ponge et al. (2002). Seasonal variations were studied in Ponge (1973). The vertical
5 distribution of the main species and their food diets were analysed in Arpin et al.
6 (1980).

7
8 In February 2004 around 300 L of soil were collected in the top 10 cm then
9 homogenized over a plastic sheet before being distributed in 10 rectangular glass jars
10 60x30x30 cm (Lxlxh). Before complete filling, two cylindrical (85x100 mm Øxh) and two
11 rectangular (75x55x105 mm Lxlxh) directional traps were positioned in each jar. The
12 traps enclosed their own volume (ca. 500 cm³) of the same soil. They were made of
13 polyvinyl chloride, one side being fitted with a 2 mm mesh plastic net, the opposite side
14 with a 20 µm mesh nylon gauze. Cylindrical traps were entirely buried into the soil, their
15 upper part (covered with either plastic net or nylon gauze) being at 1 cm from the
16 ground surface. They were positioned side by side by inverted pairs at ¼ length of the
17 glass jars, centered under the area where phenanthrene was further applied.
18 Rectangular traps were positioned side by side by inverted pairs, with their upper part 1
19 cm above the ground level, the remaining 9.5 cm being buried. The side with either
20 nylon gauze or plastic net was facing the area where phenanthrene was further
21 applied. After complete filling of the experimental jars (15 cm soil, 15 cm free space
22 above the soil), they were transported to the laboratory, then let in darkness during a
23 week in a chamber at constant temperature (20°C). This incubation period was needed
24 to let enough time for the animals to stabilize their vertical position according to species
25 requirements and to acclimate to chamber conditions.

26

27 After the acclimation period, phenanthrene was applied in five randomly
28 selected jars at the rate of 10 g per jar. The powder was applied as uniformly as

1 possible over half the surface of each jar (30 x 30 cm), thus amounting to 1.1 Kg.m².
2 Then the jars were randomly put on wood trays along both sides of the climate
3 chamber, randomization being effective on their horizontal and vertical position but also
4 on the orientation of the jars on the trays. Ten days after, the trap boxes were collected
5 then put in Berlese funnels, with the plastic net below, to extract microarthropods
6 (Edwards and Fletcher, 1971). Animals were preserved in 95% ethyl alcohol until
7 identification.

8

9 Springtails were sorted then identified at the species level using Gisin (1960),
10 Zimdars & Dunger (1994), Jordana et al. (1997), Fjellberg (1998), Bretfeld (1999),
11 Hopkin (2000) and Potapov (2001).

12

13 Prior to statistical testing, data were analysed by correspondence analysis, a
14 multivariate method which allows to discern most prominent trends in the data matrix
15 (Greenacre, 1984). Although not purposed for experimental data, this method was
16 used to avoid repeated testing of a meaningless number of variables, by focusing on
17 those species or those population parameters which responded the best to the
18 treatments. Active (main) variables were the species, none being discarded. Passive
19 (additional) variables were the treatments, and population variables such as sample
20 species richness and total abundance. Passive variables did not influence the
21 calculation of factorial axes, but they were projected among species and samples in
22 order to better interpret the significance of factorial axes. As in previous studies on
23 soil collembolan communities (Ponge et al., 2003; Gillet and Ponge, 2004), each
24 variable was standardized (mean 20, standard deviation 1) and associated to a
25 conjugate one ($x' = 40-x$) in the search for possible gradients of global abundance.

26

27 Comparisons between traps of the same jar (either on the five control or
28 phenanthrene jars) were achieved through paired t-tests, after due verification of the

1 gaussian distribution of residues. Comparisons between control and phenanthrene jars
2 were achieved through unpaired t-tests, control and phenanthrene replicates being
3 independent.

4

5 **3. Results**

6

7 Table 1 shows mean values for species abundances and population parameters
8 according to treatments. At first sight, no outward change in total abundance or species
9 richness seemed to occur within the ten days of the experiment.

10

11 The projection of active and passive variables in the plane of the first two
12 factorial axes of correspondence analysis (13.7% and 23.2% of the total variance,
13 respectively, thus totalling 36.9%) (Fig. 1) showed that the main effect of the
14 experiment was a contrast between rectangular traps used for detecting horizontal
15 movements (inwards vs outwards) and cylindrical traps used for vertical movements
16 (upwards vs downwards). This effect was trivial, since springtail assemblages of
17 rectangular and cylindrical traps differed according to behavioural requirements of
18 the species. Species which were projected on the east (positive) side of Axis 1, such as
19 *Lepidocyrtus lanuginosus* Gmelin (LLA), *Sminthurinus aureus* Lubbock (SAU),
20 *Onychiurus pseudogranulosus* Gisin (OPS), *Heteromurus major* Moniez (HMA) and
21 *Monobella grassei* Denis (MGR), could be considered typical of rectangular traps and
22 thus as living at the surface in the conditions of our experiment, while those projected
23 on the opposite side of the same axis, such as *Micranurida sensillata* Gisin (MSE),
24 *Parisotoma notabilis* Schäffer (PNO), *Isotomiella minor* Schäffer (IMI) and
25 *Pseudosinella immaculata* Lie-Pettersen (PIM) can be considered as living deeper in
26 the soil, since they were found preferentially in buried cylindrical traps. There was also
27 more specimens and more species in the traps used for capturing horizontal
28 movements than for those used for vertical movements.

1
2 However, less trivial was the difference between phenanthrene and control
3 traps which was shown on the east side of Axis 1. Treatments 'P out' (phenanthrene
4 outwards) and 'P in' (phenanthrene inwards) were projected nearer the origin of the
5 axes (barycentre) than corresponding control treatments ('C out' and 'C in'). This
6 means that phenanthrene seemed to decrease the contrast between 'horizontal' and
7 'vertical' traps. This was tested on *L. lanuginosus*, the most typical species of the
8 'horizontal' traps, which was also one of the most abundant springtail species in the
9 studied population (Table 1). *L. lanuginosus* was, as expected, more abundant in
10 'horizontal' than in 'vertical' traps, both in the presence and in the absence of
11 phenanthrene (Fig. 2), but the difference between 'horizontal' and 'vertical' traps was
12 lessened by the application of phenanthrene ($t = -3.195$, $P = 0.006$).

13
14 Axis 2 revealed an opposition between 'upwards' and 'downwards' traps, mainly
15 in the presence of phenanthrene. This was tested on *Folsomia manolachei* Bagnall,
16 which was projected far from the origin on the north (positive) side of Axis 2, more
17 remote species being rather erratic in our samples. This species was also the main
18 component of the collembolan community (Table 1). In the absence of phenanthrene,
19 no difference occurred between upward and downward trapping, while upward
20 movement was more important than downward movement in the presence of
21 phenanthrene (Fig. 3).

22 23 **4. Discussion**

24
25 Despite the absence of any bulk effect of phenanthrene, neither on total
26 abundance nor on sample species richness, some species among the dominant
27 members of the community were markedly affected by the application of phenanthrene.
28 *Folsomia manolachei*, an hemiedaphic species sensu Gisin (1943), was living mainly in

1 the first top cm of the soil in our study site (Arpin et al., 1980), but was found deeper as
2 well in our experimental conditions (Table 1). The contrast between upward and
3 downward trapping of this species, only in the presence of phenanthrene (Fig. 3),
4 showed that this species was attracted vertically to phenanthrene. This attraction did
5 not seem to proceed at large distance, since the contrast between 'upwards' and
6 'downwards' was more due to a deficit in 'downwards' specimens than to an increase in
7 'upwards' specimens (Fig. 3). Other studies showed that *F. manolachei*, as well as the
8 nearby *F. quadrioculata*, was attracted to heavy metals such as lead (Chauvat and
9 Ponge, 2002) and copper (Filser and Hölscher, 1997; Filser et al., 2000). This, together
10 with results of the present study, may explain why this species, one of the three
11 dominant springtail species in temperate soils, the other two being *Parisotoma notabilis*
12 and *Isotomiella minor*, is absent or at least poorly represented in polluted sites
13 (Bengtsson and Rundgren, 1988; Sterzynska, 1989; Sjögren, 1997; Fountain and
14 Hopkin, 2004b), although some populations may be better adapted than others
15 (Hågvar and Abrahamsen, 1990; Chauvat and Ponge, 2002). Another common
16 species, *I. minor*, did not show any trend of attraction nor of avoidance to phenanthrene
17 (Table 1). Tranvik and Eijsackers (1989) demonstrated that the absence of avoidance
18 of pollutants by this species was detrimental to its survival in a metal-polluted soil.

19

20 The effect of phenanthrene on the contrast between 'horizontal' and 'vertical'
21 traps in the other hemiedaphic *L. lanuginosus* can be explained by a decrease in
22 horizontal movements of surface-living species. In our experiment, *L. lanuginosus* was
23 living preferentially at the soil surface (Table 1, Fig. 2). The observed decrease in
24 'horizontal' minus 'vertical' densities of this species was mostly due to a decrease in
25 'horizontal' densities (Fig. 2), whether 'inwards' or 'outwards' (Table 1). Thus the main
26 effect of phenanthrene was that fewer animals were trapped when moving horizontally
27 at or near the soil surface. Since *L. lanuginosus*, as most entomobryid species,
28 disperse easily (Ojala and Huhta, 2001), we can suspect that either (i) there was a

1 decrease in the whole population due to toxic effects, (ii) animals moved more slowly at
2 the soil surface. The first hypothesis can be ruled out, since no decrease was observed
3 in 'vertical' traps. The second hypothesis is largely plausible, due to well-known
4 narcotic effects of PAHs (Still et al., 2001; Landrum et al., 2003).

5

6 **5. Conclusion**

7

8 The use of horizontal and vertical directional traps may help to better
9 understand behavioural effects of PAHs upon soil animal communities. Although no
10 effect was detected at the community level, some of the most representative species
11 responded to the application of phenanthrene at the soil surface. *Folsomia manolachei*,
12 an isotomid species, was attracted to phenanthrene, which may in the long-term cause
13 its local disappearance. *Lepidocyrtus lanuginosus*, an entomobryid species mostly
14 living at the soil surface, was mostly affected by a decrease in its dispersal rate,
15 another mechanism which may be detrimental to its long-term survival in contaminated
16 soils.

17

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19

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23

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1 **Figure captions**

2

3 **Fig. 1.** Correspondence analysis. Projection of active (collembolan species) and
4 passive (treatments, population data) variables in the plane of the first two
5 factorial axes. Variables were duplicated, except treatment variables (see text
6 for further explanation). Higher values of each variable are indicated in bold
7 roman, lower values in italic type. For the sake of clarity, only higher values of
8 springtail species are shown (lower values are symmetrical of higher values
9 around the origin of the axes)

10

11 **Fig. 2.** *Lepidocyrtus lanuginosus* in directional traps. Comparison between 'horizontal'
12 and 'vertical' traps. Values are the sum of two conjugated (inverted) boxes,
13 averaged over five replicates, with standard error of the means. Comparisons
14 between both groups were done by paired t-test. *** = significant departure from
15 null hypothesis at 0.001 level

16

17 **Fig. 3.** *Folsomia manolachei* in directional traps. Comparison between 'upward' and
18 'downward' animals trapped in 'vertical' traps. Values are means of five
19 replicates, with standard errors. Comparisons between both groups were done
20 by paired t-test. ** = significant departure from null hypothesis at 0.01 level

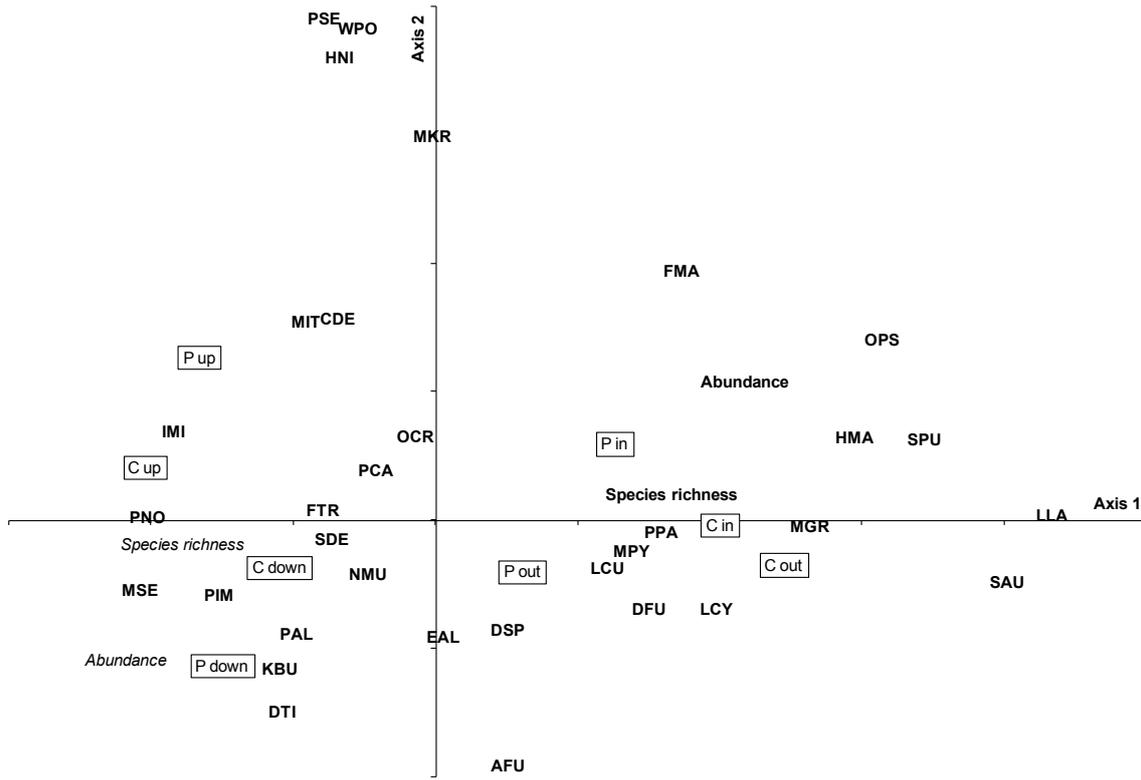
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Table 1. Mean density and species richness of Collembola in horizontal and vertical directional traps, in the presence or absence of phenanthrene. Values are means of 5 replicates, followed by standard errors

	Phenanthrene				Control			
	Inwards	Outwards	Downwards	Upwards	Inwards	Outwards	Downwards	Upwards
AFU <i>Allacma fusca</i>	0.6±0.2	1.6±0.2	0.6±0.2	0.6±0.6	0.6±0.2	1±0	0.8±0.4	0.4±0.2
CDE <i>Ceratophysella denticulata</i>	0	0	0	0	0	0	0	0.2±0.2
DTI <i>Desoria tigrina</i>	0	0.2±0.2	0.2±0.2	0.4±0.2	0	0	0	0
DSP <i>Deuterosminthurus sp.</i>	0.2±0.2	0	0	0	0	0.2±0.2	0.2±0.2	0
DFU <i>Dicyrtoma fusca</i>	1.8±0.6	1±0.4	0.6±0.2	1.4±0.7	1±0.6	3.2±0.9	1.2±0.4	1.2±0.4
EAL <i>Entomobrya albocincta</i>	0	0.2±0.2	0	0	0	0	0	0
FMA <i>Folsomia manolachei</i>	61.6±9.8	57.8±7.4	33.4±4.6	56.8±3.8	50.8±5.7	65±7.9	54.8±11.8	50.6±5.7
FTR <i>Friesea truncata</i>	4.4±1	1.8±0.2	2.6±0.5	2±0.7	2.4±1.7	2.6±0.9	2.6±0.7	3±1.3
HMA <i>Heteromurus major</i>	4±1.9	1.4±0.5	1±0.3	0.8±0.4	4±1.6	2.2±0.8	0.2±0.2	0.2±0.2
HNI <i>Heteromurus nitidus</i>	0.2±0.2	0	0	0.2±0.2	0	0	0	0.2±0.2
IMI <i>Isotomiella minor</i>	10.2±4.6	13.4±3.6	21±10.7	18.2±6.2	7.8±1.3	11.2±4.2	14.8±4.1	24.4±9
KBU <i>Kalaphorura burmeisteri</i>	0.2±0.2	0.2±0.2	2.2±0.6	0	0.4±0.2	0.2±0.2	1.2±0.5	0
LCU <i>Lepidocyrtus curvicolis</i>	0	0	0	0.2±0.2	0.4±0.2	0.2±0.2	0	0
LCY <i>Lepidocyrtus cyaneus</i>	2.2±0.9	1.6±0.9	2±0.9	0.4±0.4	1.8±0.6	1.8±0.4	0.2±0.2	0
LLA <i>Lepidocyrtus lanuginosus</i>	36.4±8.5	37.8±7.4	20.6±1.4	13.4±2.5	50.2±6.8	49.8±5.5	17.4±3.7	10.6±3.5
MIT <i>Mesaphorura italica</i>	0.4±0.2	0.6±0.4	0.2±0.2	0.4±0.2	0	0	0	0.4±0.4
MKR <i>Mesaphorura krausbaueri</i>	0.2±0.2	0.2±0.2	0	0.2±0.2	0.2±0.2	0	0.2±0.2	0
MPY <i>Micranurida pygmaea</i>	0	0	0	0	0	0.2±0.2	0	0
MSE <i>Micranurida sensillata</i>	0	0	0.2±0.2	0	0	0	0	0.6±0.2
MGR <i>Monobella grassei</i>	0.2±0.2	0.4±0.2	0	0	0.2±0.2	0.2±0.2	0	0
NMU <i>Neanura muscorum</i>	0	0	0.6±0.6	0	0.2±0.2	0	0	0
OCR <i>Oncopodura crassicornis</i>	0	0	0	0	0	0	0.2±0.2	0
OPS <i>Onychiurus pseudogranulosus</i>	10.4±2.2	7.8±1.1	3.2±0.8	4±1.2	10.2±0.9	13.2±3.7	3.2±1.2	7±3.5
PCA <i>Paratullbergia callipygos</i>	1.4±0.5	0.8±0.4	0.2±0.2	0.6±0.2	0.4±0.2	0.4±0.2	0.4±0.4	1.2±0.6
PNO <i>Parisotoma notabilis</i>	51.2±10.5	48±5.8	55.4±10.7	76.6±15.1	43.6±6.8	41.6±3.1	57.8±12.9	69±7.2
PPA <i>Pseudachorutes parvulus</i>	1.2±0.4	2±0.7	0.2±0.2	0.2±0.2	2.2±1.7	0.6±0.2	0.4±0.2	0.6±0.6
PSE <i>Pseudosotoma sensibilis</i>	0	0	0	0.2±0.2	0	0	0	0
PAL <i>Pseudosinella alba</i>	2.6±1.2	4±1.4	2.4±0.5	7.6±3.6	1.4±0.6	4.4±2.2	2.6±0.7	4.2±2.3
PIM <i>Pseudosinella immaculata</i>	0.4±0.2	0.2±0.2	0.4±0.2	0	0	0	0.6±0.2	0.4±0.2
SAU <i>Sminthurinus aureus</i>	6±0.8	4.4±1.4	2.2±1	0.8±0.4	8±1.5	9±1.4	2.4±0.5	1±0.6
SPU <i>Sphaeridia pumilis</i>	15.4±2.9	10.2±1.9	6.4±1.9	6±1.9	15.2±2	16.4±3.3	9.4±1.4	6.4±2.4
SDE <i>Stenaphorura denisi</i>	0	0.2±0.2	0	0.4±0.2	0.4±0.2	0	0.2±0.2	0.4±0.2
WPO <i>Wankeliella pongei</i>	0	0	0	0.2±0.2	0	0	0	0
Total abundance	211.2±28.7	195.8±15.7	155.6±20.7	191.6±27.6	201.4±13.3	223.4±5.5	170.8±19.6	182±22
Sample species richness	15.4±0.7	15.2±0.4	14.2±0.4	13.2±0.6	13.6±0.5	14.4±0.4	13.2±0.9	12.2±0.9

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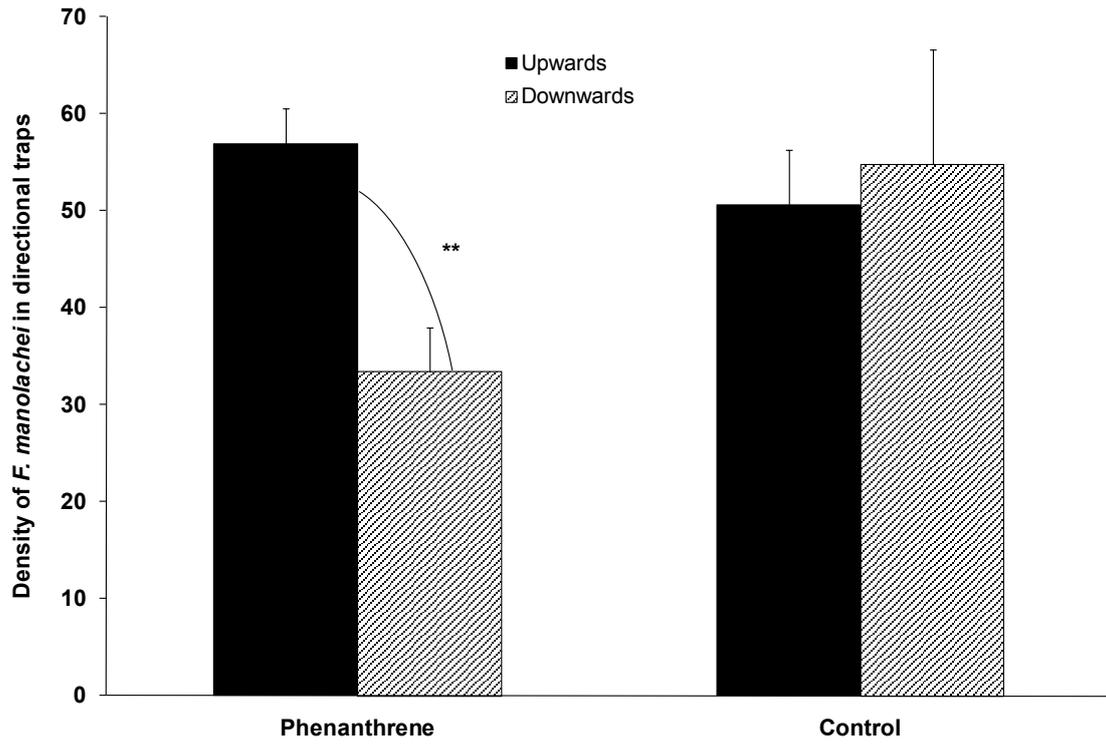
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2 Fig. 1

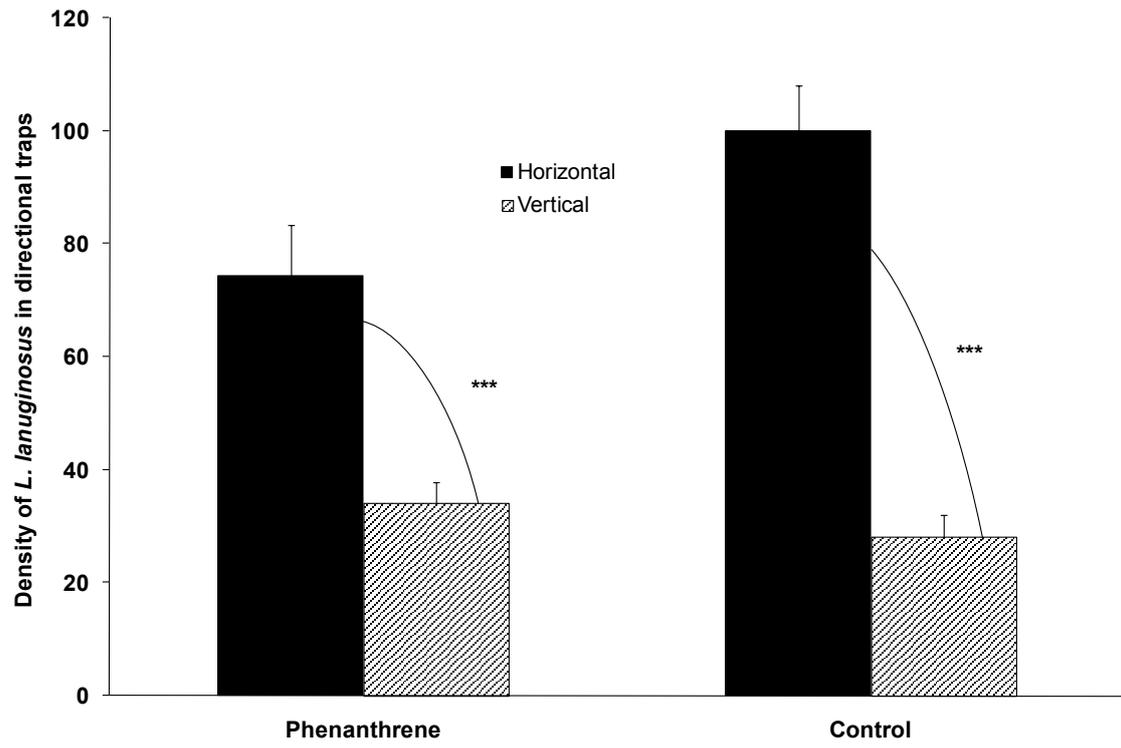
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2 Fig. 2

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2 Fig. 3