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A comparison of live versus kill pitfall traps to assess the diet of carabids through a metabarcoding approach

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Abstract

Metabarcoding approaches are powerful tools to unravel trophic relationships between predators and prey. To apply metabarcoding analyses on invertebrate gut contents, specimens must be well preserved from DNA degradation, thus the trapping method should be selected accordingly. Dry pitfall traps are commonly assumed to provide a better DNA preservation than traps that use a killing agent. However, this assumption has never been specifically tested for gut content analyses. In our study, we compared how two types of pitfall trapping, dry vs. with brine, affect the conservation of prey DNA contained in the digestive tract of predators and subsequent metabarcoding analyses. We placed dry and 'classic' pitfall traps in oilseed rape fields within an intensive agricultural area in the French Nouvelle-Aquitaine region. Traps were set up in autumn and compared for carabid trapping efficiency as well as our capacity to retrieve dietary information from the digestive tract of the main carabid species, *Nebria salina* (Fairmaire & Laboulbène) and *Calathus fuscipes* (Goeze) (both Coleoptera: Carabidae). The PCR success rate was higher in dry pitfall traps compared to classic ones for *N. salina*. We hypothesise that this was due to the presence of PCR inhibitors in the gut of this species. The ability to sequence prey DNA did not differ between specimens caught in both trap types. The list of preyed species was similar between both trap types. However, sequencing yielded more prey operational taxonomic units (OTUs) from specimens caught in dry pitfall traps, leading to differences in prey community composition and a greater ability to reconstruct prey community. Our analyses also shed light on the prey spectrum of *C. fuscipes* and *N. salina* in oilseed rape in autumn.

KEYWORDS
Calathus fuscipes, Carabidae, Coleoptera, dietary DNA, dietary information, gut contents, illumina sequencing, *Nebria salina*, oilseed rape, prey spectrum, trapping method, trophic relationship

INTRODUCTION

Carabid beetles are generalist predators and have been considered for years as important agents of biological control in agroecosystems (Thiele, 1977; Kromp, 1999; Symondson et al., 2002). In oilseed rape crops, for instance, carabids can prey on some of the major pests such as *Meligethes aeneus* (Fabricius), *Ceutorhynchus assimilis* (Paykull), and *Dasineura brassicae* (Winnertz) (Büchs & Nuss, 2000; Warner, 2001). However, measuring accurately their efficiency as pest regulator remains elusive (de Heij & Willenborg, 2020).

To study the carabid prey spectrum, large-scale temporal and spatial sampling is often conducted in order to collect a large number of specimens and allow representative coverage as well as robust statistical analysis, e.g., on seasonal dynamics of diet or the impact of some environmental parameters. Pitfall traps are typically used for the sampling of carabids and other epigeal invertebrates,

which allows estimating activity-density of various ground-active arthropod taxa such as ground beetles, rove beetles, spiders, or ants (Woodcock, 2005) or species assemblages at community level (Rainio & Niemalä, 2003). Moreover, pitfall trapping is a cheap and easy-to-use method, though leading to long-lasting debate about the capture effectiveness and the potential bias that may be encountered (Adis, 1979; Coddington et al., 1991; Woodcock, 2005; Yi et al., 2012). Although Hohbein & Conway (2018) have proposed a standard use of pitfall traps for obtaining invertebrate abundance indices, the design of the trapping method has to be adapted to the research question and the targeted organisms. Consequently, much variation in the trap design, material, colour, size, use of funnels and rain guards, and duration have been noted (Weeks & McIntyre, 1997; Brown & Matthews, 2016). One additional point of variation, which has caused much debate, is the use of a killing agent inside the trap.

Indeed, in order to minimise the financial costs and logistic effort of sampling, various killing agents have been tried (Hohbein & Conway, 2018), i.e., formalin, picric acid, or ethylene glycol. These are now rarely used due to hazardous effects on human health or wildlife (Hall, 1991). More recent agents, such as propylene glycol, ethanol, brine (at varying concentrations), or water were compared for their efficiency to capture rate (Curtis, 1980; Topping & Sunderland, 1992; Koivula et al., 2003; Schmidt et al., 2006), morphological preservation (Sasakawa, 2007; Braun et al., 2009; Aristophanous, 2010), and DNA preservation (Gurdebeke & Maelfait, 2002; Vink et al., 2005).

The choice of preservatives is particularly crucial when the trapped samples are to be used for subsequent genetic analysis and, most importantly, molecular sequencing. For optimal results, molecular methods require specimens in which the DNA is well-conserved. Consequently, the main criteria when selecting a sampling method for such studies is to protect the DNA from chemical and enzymatic degradation. Similarly, conservation methods used subsequently to the trapping, can also cause DNA degradation, for example the hydrolytic and oxidative effects of ethanol, especially at low concentration and high temperature (Vink et al., 2005). Freezing or drying insect specimens are the most suitable methods to preserve the DNA in a sample (Post et al., 1993; Reiss et al., 1995). However, these are difficult to apply in the field.

Potential strategies to limit the DNA degradation include using a killing agent that limits DNA degradation during the sampling period (Reiss et al., 1995; Stoeckle et al., 2010; Pokluda et al., 2014) and/or reducing the duration of the sampling. When the duration of the sampling is greatly reduced, it is possible to operate without preservatives and thus use dry pitfall traps, in which the killing agent is replaced by a non-lethal substrate and the trapped specimens stay alive until they are picked up. Such traps are often preferred when specimens are used for metabarcoding analysis of carabid's gut contents (e.g., Roubinet et al., 2017; Kamenova et al., 2018a; Frei et al., 2019), especially because they allow to obtain regurgitates, which give a higher prey DNA detection success for large amplicons (Waldner & Traugott, 2012). Because dry pitfall traps avoid the samples being submerged in a trapping agent that is potentially deleterious for the prey DNA contained in the gut, we may assume that prey DNA inside the gut of predators is better preserved in those traps. However, by keeping predators alive, the enzymatic reactions inside their digestive tract are maintained after trapping and this could lead to greater degradation of prey DNA. Therefore, it remains unknown whether dry traps affect our ability to retrieve robust information about prey–predator interactions.

Our aim here was to compare two types of pitfall trapping, dry vs. with brine (here called 'classic'), regarding the conservation of prey DNA contained in the digestive tract of predatory species and subsequent analyses. To do this, we used a field-based approach together with a metabarcoding approach to analyse the diet of carabids captured in 21 oilseed rape fields with either dry or classic traps, and compared the results to assess the impact of the trapping method on our capacity to retrieve dietary information from metabarcoding.

MATERIALS AND METHODS

Carabid collection and sample processing

We captured carabid beetles from 21 oilseed rape crops located in an intensive agroecosystem within the Long-Term Social-Ecological Research *Zone Atelier* 'Plaine & Val de Sèvre' (hereafter ZAPVS, Nouvelle-Aquitaine; see Bretagnolle et al., 2018a). Samples were collected in autumn, during two sampling periods in October 2020 (5–9 and 26–30). In each field, 10 pitfall traps (Figure 1A) were used, five were dry (half filled with clay beads) and five were classic pitfall traps (half filled with 200 mL of water, 10 g L^{-1} of salt, and five drops L^{-1} of odourless soap). The classic pitfall trap used here is the main type of trap used for biodiversity monitoring in the ZAPVS (Bretagnolle et al., 2018b). The traps were positioned on two lines in a staggered arrangement (Figure 1B). Because 90% of carabid species reproducing in autumn are active at night (Thiele & Weber, 1968), the traps were activated for 1 night between 17:00 and 08:00 hours the next day (so, maximum 15 h).

Each pitfall trap consisted of a 1-L plastic cup associated with a funnel (125 mm at the wide end and 15 mm at the narrow end) buried in the ground with the lip of the funnel flush with the soil surface. A plastic plate provided shade and protection from rain (Figure 1A).

All trapped invertebrates inside the dry pitfall traps were collected in the morning, placed in a coolbox for transport, and stored at –20 °C within 3 h. After 2–3 h of freezing, carabid specimens were sorted, identified and sexed, and placed individually in 1.5-mL tubes filled with

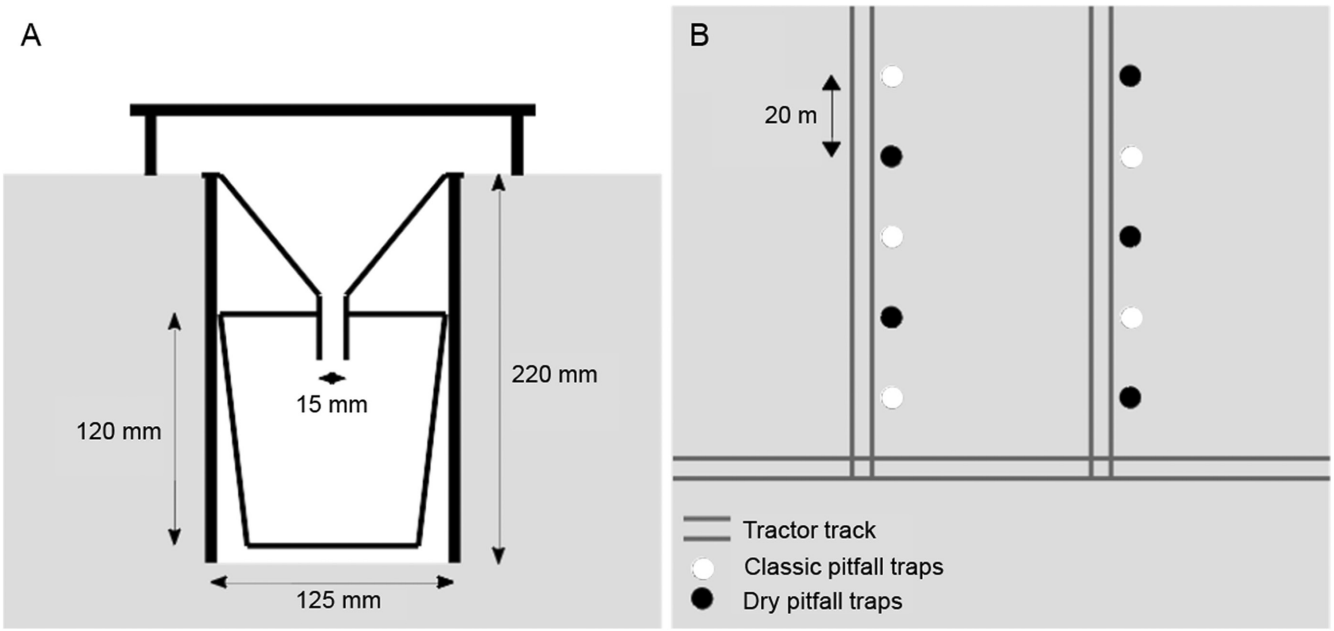

FIGURE 1 (A) Pitfall trap design. Traps were filled to half the height with salted water and odourless soap (classic trap) or with clay beads (dry trap). (B) Pitfall traps staggered arrangement in the field. The parallel tractor tracks are separated by approximately 40 m.

300 μL of 100% ethanol. Carabid specimens trapped in classic pitfall traps were first sorted, quickly dried on absorbent paper, identified and sexed, and also placed individually in 1.5-mL tubes with 300 μL of 100% ethanol. All samples were stored at −20 °C until subsequent processing.

Out of 611 captured specimens, 292 were used for the gut-content extraction (162 trapped with classic pitfall traps and 130 with dry pitfall traps). Selection was based on molecular diet analysis goals, that was only conducted on specimens belonging to the dominant carabid species (i.e., those representing >10% of trapped individuals). In addition, to maximise representativeness of the various traps and the different fields, no more than eight individuals per trap and 30 individuals per field were kept for subsequent analyses. Gut contents were isolated by the dissection of the crop, a bulb-like organ situated between the oesophagus and the proventriculus. This part of the foregut is particularly suitable for the study of the diet because it is a mechanically functioning unit ensuring filtration and food storage (Jaspar-Versali et al., 1987) and because it is made of a thick epithelium, which makes it more easily extractable than the other parts of the digestive tract. Dissections were performed with sterilised forceps. Between two samples, forceps were successively dipped in bleach, Decon 2%, MicroBeads sterilizer (300 °C; Fisher Scientific, Loughborough, UK), and then sprayed with 96% ethanol. This sterilisation process lasted about 45 min before the same forceps was used again for another sample. Two forceps were used for the dissection and a third one was exclusively used to harvest the crop without touching the outer surface of the beetle.

The dissected crop was emptied in 300 μL of lysis buffer from the NucleoMag Tissue kit for purification from cells and tissue (Macherey-Nagel, Düren, Germany). The emptied crop was discarded to limit the carry-over of predator DNA in the gut content. At this point, samples were chosen in order to avoid specimens with visually empty crops. For all other specimens, gut content samples were stored at −20 °C until further processing.

Library preparation and sequencing

DNA from gut content samples was extracted using the epMotion 5075 workstation (Eppendorf, Hamburg, Germany) and the NucleoMag Tissue kit for purification from cells and tissue (Macherey-Nagel) following the manufacturer's protocol. Initial gut content solution volume was 250 μL, the final elution was performed in 100 μL and the DNA extract was stored at −20 °C until PCR amplification.

A 313 bp fragment of the mitochondrial cytochrome oxidase I (COI) gene was amplified using the generic primer pair mlCOIintF (5′-GGWACWGGWTGAACWGTWTAYCCYCC-3′) and jgHCO2198 (5′-TAIACYTCIGGRTGICCRAARAAYCA-3′) (Folmer et al., 1994; Leray et al., 2013), correctly modified for high-throughput sequencing (HTS) on Illumina. PCR amplification reactions (25 μL) contained the following: 2 μL of template DNA, 1 μL of each primer (10 μM), 5 μL of 5X GoTaq (Promega) reaction buffer, 1 μL of $MgCl_2$, 1 μL of BSA, 0.5 μL of dNTPs, 13.375 μL of molecular-grade water, and 0.125 μL of GoTaq G2 polymerase (Promega). PCR conditions were: 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 1 min, annealing at 48 °C for 1 min, and elongation at

72 °C for 1 min 30 s, then a final elongation step was performed at 72 °C for 5 min. Amplification success was checked through a 1% gel electrophoresis. Amplified amplicons were purified with a NGS clean up and size selection kit (Macherey-Nagel) following the manufacturer's protocol. The final elution was performed in 40 μL.

Later, the COI metabarcoding library was prepared by ligating Nextera XT indices through a 10-cycle PCR (with the same conditions as for the initial PCR except for the annealing temperature which was 53 °C for this second PCR). The concentration of the successfully ligated samples (checked on 1% agarose gel) was measured using a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA). Samples were then pooled in equimolar proportions (40 ng each), selected by size in a 1% agarose gel and purified using the GeneJet Gel Extraction kit (Life Technologies), according to manufacturer's protocol and the pools eluted in 30 μL. Purified pools were combined into a 40-μL final pool (4 nM). Sequencing runs were carried out on an Illumina Miseq using V2 chemistry (2 × 300 bp, 500 cycles) at the Sequencing Centre within the Biozentrum of the Ludwig-Maximilian-University in Munich (Germany). The raw dataset generated and analysed during the current study has been submitted to the NCBI Sequence Read Archive (SRA) under the BioProject PRJNA874542.

COI metabarcoding library filtering and taxonomic assignment

The FastQC software (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used to check the quality of the libraries (demultiplexed fastq files) on forward and reverse reads. Primer pairs were removed using *cutadapt* (Martin, 2011) and reads were merged with PEAR (Zhang et al., 2014), setting Phred score 30 as a threshold. The subsequent quality filtering (fastq_maxee = 1), dereplication, denoising, insertion and deletions (indels), and chimera removal were performed using the Vsearch v.2.8.2 software (Rognes et al., 2016), which produced a fasta file containing Amplicon Single Variants (ASVs). These ASVs were clustered into operational taxonomic units (OTUs) applying the centroid-based greedy clustering algorithm with a cut-off threshold of 97% (Xiong & Zhan, 2018) and an OTU table was mapped also using Vsearch v.2.8.2 software (Rognes et al., 2016). Taxonomic classification of the sequences was performed against NCBI Genbank nr/nt using the BLAST algorithm (Johnson et al., 2008) and R package *Taxonomizr* (https://github.com/sherrillmix/taxonomizr/), in order to infer the species level classification when possible.

Operational taxonomic units with an identity <85% or a query coverage <95% were considered as not assigned. Those with an identity of 85–93% were assigned to family, those of 93–97% to genus, and OTUs with >97% identity were assigned to species level.

Data analysis

The impact of trap type and sampling session on capture rate was analysed using generalized linear mixed effect models (GLMM) with Poisson distribution using the package *lme4* (Bates & Maechler, 2013). In this model, field ID was considered as a random effect. Likewise, the potential sex ratio differential between traps was assessed with a GLMM with binomial distribution with trap type and carabid species as fixed effects, and field ID and session as random effects.

To explore the trap type influence on the PCR success, we computed a binomial GLMM with trap type and species as explanatory variables. Field ID and session were considered as random effects. PCR success was defined by the presence/absence of visible amplified DNA band on the electrophoresis gel after 40 cycles of COI amplification and 10 cycles to attach the indices. A binomial GLMM was then performed on each species individually.

We estimated the completeness of the sampling by computing a rarefaction curve using R package *vegan* (Oksanen et al., 2013). The cumulative numbers of OTUs detected from individuals trapped in the classic pitfall traps and the dry pitfall traps for the two species were compared using a Kolmogorov–Smirnov test.

Next, the diet of carabids in oilseed rape crops was described using the frequency of occurrence (FOO) of prey OTUs, which informs about the number of samples (counts) in which an OTU is present (Deagle et al., 2019). Only occurrences with at least two reads were considered. Relative read abundance (RRA), the proportion of reads of each OTU (Deagle et al., 2019), was not analysed in this study, as most of the reads were predator reads whereas we were interested in prey. From the raw data, reads corresponding to carabid OTUs were thus discarded, as it was not possible to discriminate between the DNA of the predators themselves and that of other carabids which may have been preyed on. Contaminant OTUs were also discarded (fungi, algae, and taxa for which identity, prey, or contaminant cannot be decided). The FOO for prey OTUs was calculated using customized scripts using R package *dplyr* (Wickham et al., 2019).

To test the influence of the trap type on the sequencing results, we performed GLMM with Poisson distribution on the number of prey OTUs per carabid specimen, and a GLMM with binomial distribution on the sequencing success (as the presence/absence of at least one prey OTU per carabid specimen). Trap type and species were considered as fixed effects and field ID and the session as random effects.

Prey communities (beta diversity) of carabids caught between the two trap types, were compared using a non-permutation multivariate analysis (PERMANOVA) with 999 iterations on the beta diversity using R package *vegan* (Oksanen et al., 2013). All statistical analyses were performed with RStudio (R v.4.2.1).

RESULTS

Trapping efficiency

In total 611 carabid specimens from 14 carabid species were collected in oilseed rape fields, most of which (94.2%) belonged to two carabid species, *Nebria salina* (Fairmaire & Laboulbène) (n = 458) and *Calathus fuscipes* (Goeze) (n = 115) (Table 1). The other species were represented by a maximum of six individuals over the two sampling periods. Mean (± SE) species richness was 0.84 (± 0.78) in classic traps and 0.78 (± 0.72) in dry traps. These low diversity values do not allow to do a proper comparison of the two trap types in this respect. The densities-activities were 1.78 (± 2.68) and 1.72 (± 2.00) in classic and dry pitfall traps respectively. We found an effect of the interaction between trap type and sampling session on the capture rate (t = –2.06, d.f. = 60, P = 0.044) but no effect of the trap type alone (t = 1.31, d.f. = 60, P = 0.20). Carabid density-activity was indeed higher in classic pitfall traps during the first sampling session whereas it was higher in the dry traps during the second sampling session (Table 1). Capture rate showed a strong bias in sex ratio: of the 292 individuals used for the gut contents analysis, 215 were males and 77 were females (*N. salina*: 172 ♂, 55 ♀; *C. fuscipes*: 43 ♂, 22 ♀) but this bias was not related to the trap type (z = –1.04, P = 0.30).

PCR success

Out of 292 gut samples, 249 were successfully amplified for COI (187 *N. salina* and 62 *C. fuscipes*). PCR success rates were 92.3% in dry traps and 79.6% in classic ones. We found a significant effect of the species identity on PCR success (z = –2.07, P = 0.038). We found a higher PCR success with *C. fuscipes* specimens (95%) compared to *N. salina* specimens (82%). Performed separately on each species alone, PCR success was higher with *N. salina* individuals caught in dry pitfall traps than with individuals trapped in classic pitfall traps (91.2 vs. 75.2%; z = 2.139, P = 0.032). In contrast, there was no difference between trap types in *C. fuscipes* (classic 94.6%, dry 96.4%; z = 0.34, P = 0.74).

Sequencing success

DNA was successfully sequenced from 97.2% of the samples. In total 14 261 981 reads were obtained after clean-up. On average (± SE), 45565 reads (± 17054) were obtained per sample. The vast majority of those reads corresponded to carabids (95%). The remaining reads corresponded to prey (2.7%), fungi (1.2%), algae (0.6%), other contaminants (0.2%), and non-assigned OTUs (0.3%).

The OTU accumulation curve suggested that the diet of the studied species was well estimated by our analysis with 82.4% of OTUs detected for *N. salina* and 89.9% for *C. fuscipes* (Figure 2). There were clear differences in the cumulative number of OTUs detected from individuals trapped with classic and dry pitfall traps for *N. salina* (Kolmogorov–Smirnov test: D = 0.35, P < 0.0001) and *C. fuscipes* (D = 0.56, P < 0.0001). Based on rarefaction curves, dry traps retrieved more OTUs than the classic traps for both *N. salina* and *C. fuscipes*.

With regard to taxonomic assignation, 258 OTUs were assigned to contaminants. Ninety-five OTUs were assigned to the Carabidae family, including 42 OTUs assigned to *N. salina* and *Nebria* sp., 33 to *C. fuscipes* and *Calathus* sp., four to Carabidae sp., and finally 16 OTUs were assigned to other carabid species [*Amara consularis* (Duftschmid), *Anchomenus dorsalis* (Pontoppidan), *Harpalus rufipes* (De Geer), *Harpalus dimidiatus* (P. Rossi), *Poecilus cupreus* (L.), and *Zabrus tenebrionides* (Goeze)]. The remaining 142 OTUs matched prey DNA but 67 of them (representing only 237 reads) were discarded because they comprised only one read. Out of the 75 remaining prey OTUs, 68 were found in *N. salina* samples (44 in classic and 52 in dry pitfall traps) and 19 in *C. fuscipes* samples (11 in classic and 13 in dry pitfall traps), with 12 OTUs in common between the two species. Based on their percentage of identity, 38 prey OTUs were assigned to species level, 15 to genus level, and 22 to family level.

Prey OTU were found in 134 *N. salina* individuals out of 190, and 41 *C. fuscipes* individuals out of 59 (Figure 3). There were, on average, 1.75 prey OTUs per individual in *N. salina* (classic: 1.84, dry: 1.65) and 1.36 in *C. fuscipes* (classic: 1.35, dry: 1.36). There was no influence of trap type on the sequencing success (z = 0.22, P = 0.83). Likewise, the number of prey OTUs per sample did not differ between individuals

TABLE 1 Carabid density-activity and species richness recorded in classic (n = 105) and dry (n = 105) pitfall traps set up in 21 oilseed rape fields, with a focus on the dominant species *Nebria salina* and *Calathus fuscipes*.

Trap type	Session 1 (5–9 Oct 2020)				Session 2 (25–29 Oct 2020)			
	Density-activity				Density-activity			
	All carabids	*N. salina*	*C. fuscipes*	Species richness	All carabids	*N. salina*	*C. fuscipes*	Species richness
Classic	151	98	43	8	154	134	14	6
Dry	103	51	39	10	203	175	19	7
All traps	254	149	82	12	357	309	33	9

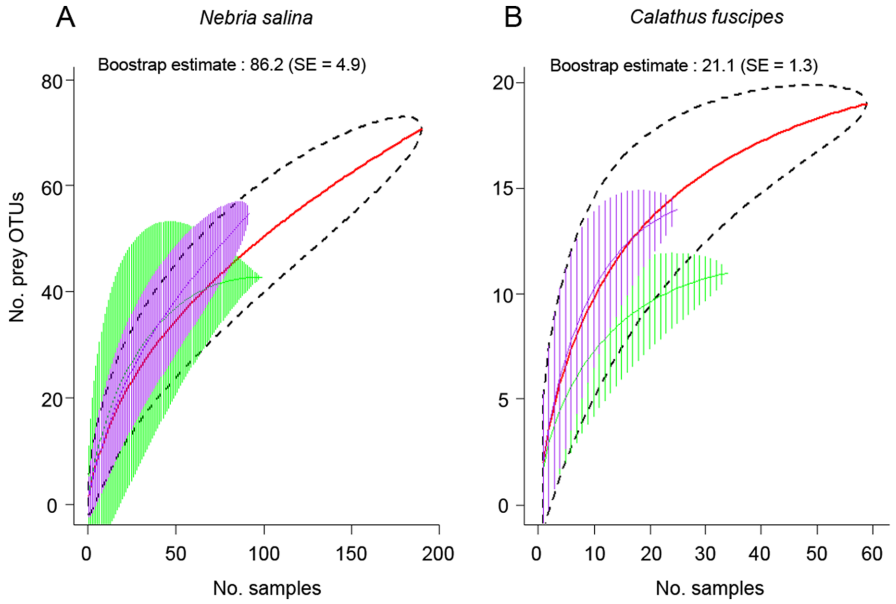

FIGURE 2 Detection of prey operational taxonomic units (OTUs) in predator gut contents: cumulative curves of number of prey OTUs in relation to number of samples for the carabid beetles (A) *Nebria salina* and (B) *Calathus fuscipes*. The green and purple solid lines represent the estimated total number of prey OTUs in the carabid diets obtained with classic and dry pitfall traps, respectively; the hatched areas correspond to cumulative curves. The red solid lines are based on all traps; the areas delimited by the dashed lines correspond to the 95% confidence intervals.

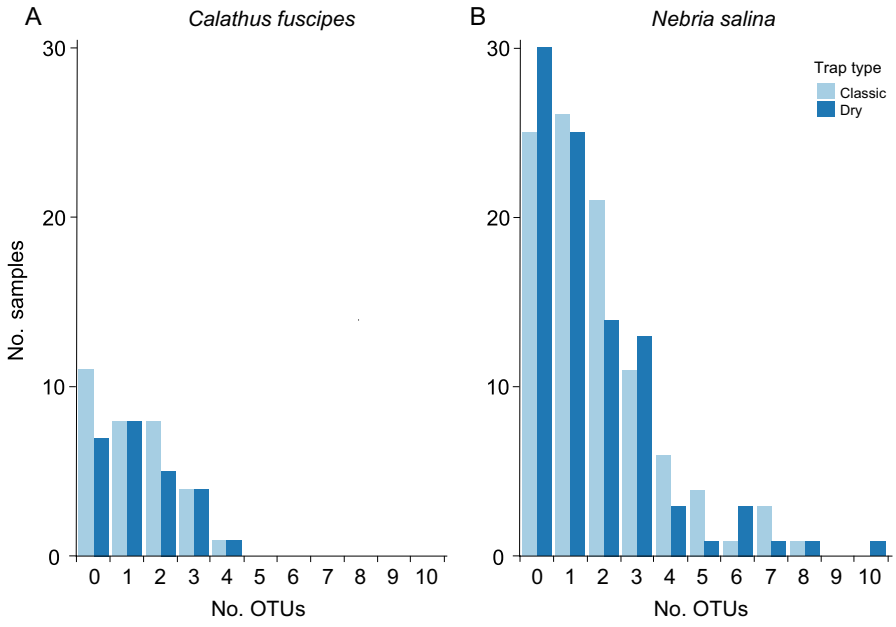

FIGURE 3 Distribution of specimens of the carabid beetles (A) *Calathus fuscipes* and (B) *Nebria salina*, collected in classic and dry pitfall traps, according to the number of prey operational taxonomic units (OTUs) they contained.

trapped in the two trap types for *N. salina* and *C. fuscipes* ($z = 0.1$, $P = 0.62$).

For *N. salina*, we found 15 invertebrate orders (14 in classic and 13 in dry pitfall traps) (Figure 4A,B). However, the frequency of occurrence was different for the two trap types. The main prey orders for samples trapped in classic pitfall traps were Entomobryomorpha (4 OTUs; FOO = 43%), Opisthopora (5 OTUs; 24%), and Diptera (8 OTUs; 14%), whereas the main prey orders for the samples trapped in dry pitfall traps were Opisthopora (10 OTUs; 27%), Diptera (18 OTUs; 22%), and Entomobryomorpha (3 OTUs; 22%).

For *C. fuscipes*, we found 10 invertebrate orders (nine in classic, seven in dry pitfall traps; Figure 4C,D). The relative contribution of each order in the diet of the specimens trapped in classic and in dry pitfall traps was similar. There was a major contribution by Opisthopora [especially by *Aporrectodea longa* (Ude), Lumbricidae] present in 44% (classic) and 36% (dry) of the samples and by Araneae [only *Amaurobius erberi* (Keyserling), Amaurobiidae] present in 38 and 32% of the samples.

When analysing the beta diversity, we found a difference in the prey OTUs composition for carabids trapped in classic and dry pitfall traps for *N. salina* (PERMANOVA, $F = 1.46$, $R^2 = 0.01$, $P = 0.047$), but not for *C. fuscipes* ($F = 1.06$, $R^2 = 0.026$, $P = 0.35$).

DISCUSSION

Metabarcoding approaches are powerful tools for the characterization of food webs (Pompanon et al., 2012). The diet of a high number of specimens can be characterized

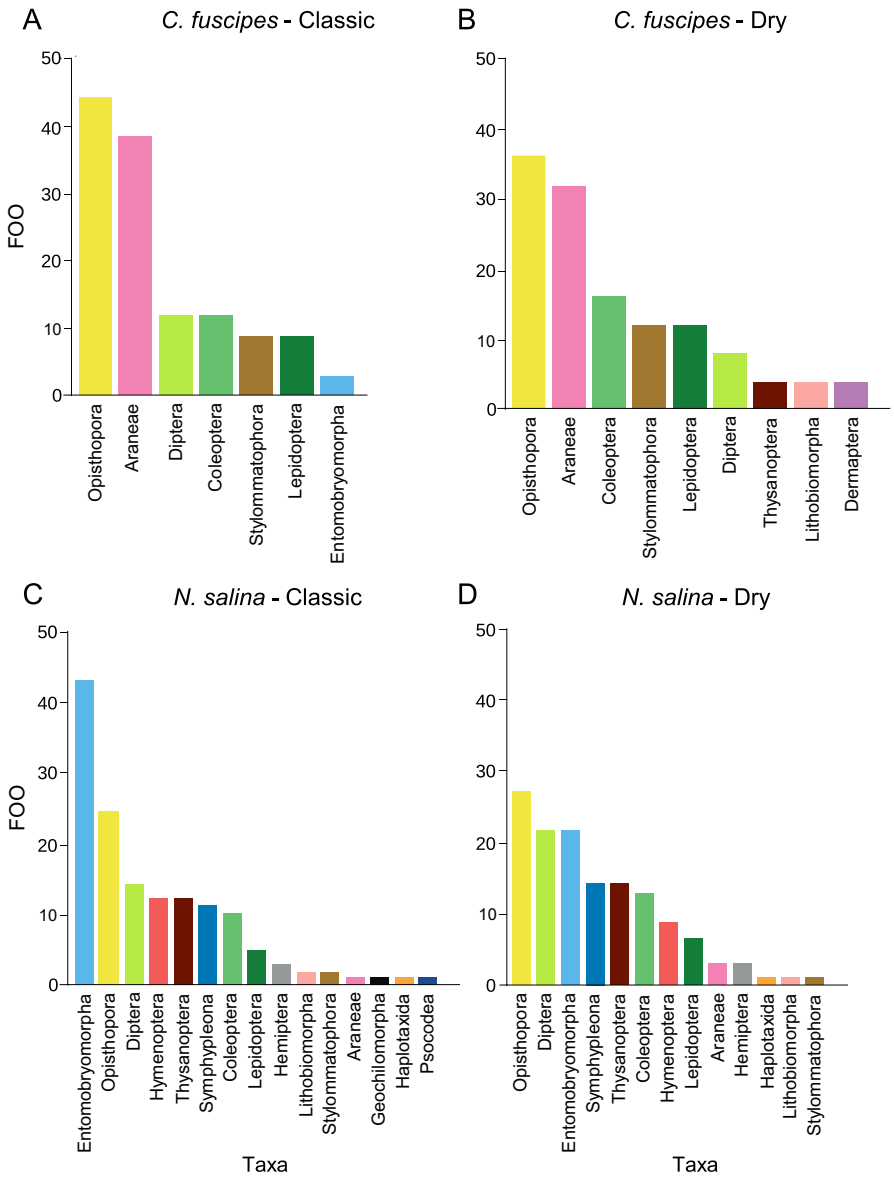

FIGURE 4 Frequency of occurrence (FOO) of the main invertebrate orders in the diet of (A, B) *Calathus fuscipes* and (C, D) *Nebria salina*, captured in oilseed rape fields with (A, C) classic and (B, D) dry pitfall traps. Diet of (A) 24 individuals comprising 11 prey operational taxonomic units (OTUs), (B) 18 individuals comprising 13 prey OTUs, (C) 74 individuals comprising 43 prey OTUs, and (D) 61 individuals comprising 55 prey OTUs.

in a unique sequencing run and in a cost-effective manner, without the need to estimate precisely the diet composition prior to performing the study (De Sousa et al., 2019). When applied on an arthropod community in the context of an agroecosystem, metabarcoding offers the potential to retrieve the trophic relationships between predators and prey (Sow et al., 2020; Cuff et al., 2021) or between parasitoids and hosts (Sigut et al., 2017; Sow et al., 2019), and subsequently to assess pest regulations and identify promising biocontrol agents. However, the use of such methods or techniques is dependent on the quality of DNA, and therefore relies on its (hopefully low) degradation in samples (Deagle et al., 2006). Improving knowledge about insect trapping may help to lessen DNA degradation and, therefore, improve our understanding of food networks within agroecosystems. When comparing the performance of dry vs. 'classic' pitfall traps, we found that the trap type did not have any effect on carabid species richness and prey OTUs sequencing. In both trap types, the two most trapped species were *N. salina* and *C. fuscipes*, representing ca. 94% of the carabids trapped. The PCR success rate, however, was higher in dry pitfall traps than in classic ones for *N. salina*, but no difference was found for *C. fuscipes*. Moreover, dry traps allowed for more effective prey detection. Below, we discuss our results and propose methodological improvements for carabid trapping in agroecosystems.

Trapping efficiency

We found that the density-activity of carabids was higher in classic pitfall traps during the first sampling session whereas it was higher in the dry traps during the second session. Indeed, the number of trapped carabids differed between the trapping session thanks to dry pitfall traps which captured twice as many individuals during the second session compared to the first, whereas the classic

traps captured about the same number. This difference came especially from the increase in the density-activity of *N. salina* which did not compensate for the decrease in that of *C. fuscipes* between the two sampling sessions. This differential trapping can be due to the influence of the capture rate of two classic traps in one of the fields during the first sampling session (with 41 specimens trapped, i.e., 27% of the whole sampling by the 105 classic traps).

The number of carabid species collected per trap was very low, probably because traps were only activated for 1 night. Thus, testing whether the two trap types are equally effective to capture carabid species was not possible using our data. However, the numbers obtained do not suggest any striking difference between the two trap types in terms of species diversity, which contrasts with a previous study in which a higher species richness (but not higher abundance) of trapped specimens was recorded in killing pitfall traps compared to dry ones (Weeks & McIntyre, 1997). The lack of differences in species richness between the two trap types may be explained by the fact that we restrained the sampling to carabids and did not consider all arthropod species as did Weeks & McIntyre (1997). Furthermore, the use of a killing agent may have impacted the activity-density and diversity observed in the latter study due to increased local humidity or attractive odour sources (Woodcock, 2005). Indeed, we took care by removing odours in our classic traps (odourless soap, beads, and traps cleaned after each use). We noted a strongly biased sex ratio towards males in both species and trap types, which may result either from higher dispersal activity by males especially when searching for females for mating purpose, or from lower activity by females especially when satiated (Wallin & Ekbom, 1994; Szyszko et al., 2004).

A major limitation of the dry trapping method is the potential predation inside the trap (Woodcock, 2005; McKravy, 2018). However, few studies have attempted to estimate this (Mitchell, 1963; Roubinet et al., 2017) and most authors conclude that it is an infrequent phenomenon (e.g., identified in four traps out of 100 by Roubinet et al., 2017). Here we used clay beads in the dry pitfall to limit the encounters between captured prey. During our sampling sessions, the insects' parts (e.g., wings, legs, antennae), which were sometimes found inside the trap seemed not to be the result of a predation, but rather the result of the perturbations which have occurred during the transport (between the field and the laboratory) and the freezing of specimens prior to sorting.

PCR success

PCR success was lower for *N. salina* collected with classic pitfall traps compared with those from dry pitfall traps. Upon dissection, carabids' crops were examined and we discarded samples where no leftover food was visible. Thus, empty crops cannot be the reason for the PCR failure. The killing agent, as used in classic traps, can have deleterious effects on the gut contents (Schmidt et al., 2006; Szinwelski et al., 2012). Ethanol has hydrolytic and oxidative effects on DNA (Vink et al., 2005). The killing agent can also act as an inhibitor leading to failed PCR during library preparation, such as formaldehyde (Gurdebeke & Maelfait, 2002) or salt (Davalieva & Efremov, 2010), which was used in our classic traps. Specimens from classic traps could have swallowed salt water, which was then mixed with the crop contents. Moreover, pieces of soil and other fragments of vegetation, which are often found in traps, could bring other PCR inhibitors, such as humic acid (Watson & Blackwell, 2000; Schrader et al., 2012) and plant polysaccharides (Demeke & Adams, 1992; Schrader et al., 2012). *Nebria salina* is a smaller species than *C. fuscipes* and may have been more impacted by this issue. Even if prey DNA was totally degraded inside the crop, predator DNA is always collected with the gut contents and ensures PCR success with COI primers (especially because the carabid DNA is in good condition compared with the prey DNA). The fact that several PCRs failed, means that even the carabid DNA could not be amplified, which supports the inhibitor hypothesis. We can also hypothesize that the difference between *N. salina* and *C. fuscipes* is linked to the diet of *N. salina*. Although we only amplified animal prey DNA, we could observe during dissections the presence of plant remains in the crops of *N. salina* but not in those of *C. fuscipes*. Kamenova et al. (2018a) also showed that *N. salina* frequently consume plant material. The presence of plant material in the diet of *N. salina* is likely to enrich their crop contents with PCR inhibitors (Demeke & Adams, 1992; Schrader et al., 2012).

Sequencing success

Conversely to PCR, sequencing success (taken as the ability to retrieve at least one prey OTU) was not different between carabids caught in the two trap types. Likewise, the number of prey OTUs was not different between individuals caught in the two trap types. When we analysed the beta diversity, we did not find a difference in prey OTUs composition between both trap types for *C. fuscipes* but a slight difference for *N. salina*.

For *C. fuscipes*, the two major prey, *A. erberi* (a spider) and *A. longa* (a worm), could be detected equally well and with the same rank order in the two trap types. For *N. salina*, the three main prey orders were always Opisthopora, Diptera, and Entomobryomorpha, regardless of the type of trap. Only the frequencies of occurrence differed. The more notable difference concerns Entomobryomorpha which were detected in 43% of individuals trapped with classic pitfall traps but only in 22% of individuals trapped with dry ones. This overrepresentation may result from the degradation of prey DNA during the carabid digestion, which continues in dry pitfall traps until the specimens are frozen. For medium-sized DNA fragments (300–500 bp), the detectability half-life (T_{50}, the estimated time post-feeding

for a 50% prey DNA detection probability) ranged around 30 h for carabids in Waldner et al. (2013) or around 40 h in Kamenova et al. (2018b). Traps were activated for 15 h at the most to limit prey digestion by the predators. However, it is likely that carabids had consumed one or more prey potentially hours before falling into the trap, which increased the risk that prey DNA had been digested and could not be detected. On the contrary, in classic traps, although digestion may continue for some time, degradation is probably slowed down. It seems that this problem was not acute in our case as accumulation curves and the prey OTUs numbers actually indicated that dry pitfall traps make it possible to reconstitute more effectively the prey community than classic pitfall traps.

As opposed to specimens trapped with concentrated ethanol as killing agent (Szinwelski et al., 2012), those collected in standard traps containing slow-killing agents (such as water or brine) can react with regurgitations or defecations (King et al., 2008) or by swallowing liquids. These reactions may cause cross contamination among the trapped specimens. Athey et al. (2017) on the contrary showed that a slug drowned inside ethanol for 24 h did not contaminate the gut contents of the carabid *Pterostichus melanarius* (Illiger). Moreover, they did not detect any amplifiable slug DNA in the killing agent. Conversely, Shokralla et al. (2010) showed that even in absence of regurgitation or defecation, specimens start diffusing amplifiable DNA into ethanol after just 24 h. It is not excluded that this killing agent can be a source of cross contamination, especially if many specimens are mixed in the liquid. This is probably the case with Collembola, which were abundantly trapped. Hence, the overrepresentation of Entomobryomorpha DNA in specimens trapped in classic pitfall traps could be the consequence of cross contamination between Collembola and carabids soaking in the same liquid.

A small number of reads came from prey OTUs in comparison to reads coming from carabid OTUs. Because of the phylogenetic proximity between the predator and its prey, the PCR with universal primers leads to amplification of both the prey and the predator (O'Rorke et al., 2011; Piñol et al., 2013). Therefore, the competition with the predator DNA can prevent the amplification of prey DNA (Shehzad et al., 2012; Piñol et al., 2013). This situation is even more acute with a high degradation of prey DNA in the gut (Cuff et al., 2022). On the contrary, the predator DNA is generally in good condition, and despite the precautions taken during the dissection, it can remain in high quantity inside the sample as compared to prey DNA. Therefore, more predator DNA is amplified during the PCR compared to prey DNA. One solution could be to develop blocking primers to prevent the amplification of predator DNA (Vestheim & Jarman, 2008). However, developing effective blocking primers is not easy, especially for carabids (Kamenova, 2013).

An alternative solution is to lower the detection threshold to avoid rejecting any prey DNA occurrence and retrieve as much dietary information as possible. When following this strategy, prey OTUs are represented by low numbers of reads, and it can be difficult to distinguish data output from potential contamination (Drake et al., 2022). That is why special attention has been taken to prevent contamination between samples or with the laboratory equipment during the sample preparation for sequencing. In our analysis, no prey DNA was detected from the blanks, suggesting that the contamination risks were properly mitigated.

Prey spectrum of *Nebria salina* and *Calathus fuscipes* in autumn

These two species are well known to be autumn breeders (Holland, 2002), active at night (Kegel, 1990; Bargmann et al., 2016) and considered as generalist predators (Luff, 2002; Bargmann et al., 2016). Their diet is not well known, and therefore our study provides key information about their prey spectrum in autumn. *Nebria salina* is known to consume plants, Diptera, and Araneae in wheat and oilseed rape fields (Kamenova et al., 2018a). The species is morphologically very similar to *Nebria brevicollis* (Fabricius), whose diet is more often studied and seems mainly composed of Collembola and mites, along with other soil-living arthropods as accessory food (Penney, 1966; Toft & Bilde, 2002). In oilseed rape, *N. salina* showed here a clear preference for decomposers, Collembola, mainly Isotomidae (Entomobryomorpha) and Sminthuridae (Symphypleona), and earthworms (Opisthopora, Lumbricidae). Collembola is one of the most abundant groups of arthropods in agrosystems and recognized as a major prey group for carabids (Mitchell, 1963; Hengeveld, 1980; Pollet & Desender,1987). These organisms are generally considered as a low-quality food for adult carabids (Bilde et al., 2001) and are not easy prey (due to their escaping abilities), which has led certain predatory species to develop specific morphological adaptations to hunt Collembola (Baulechner et al., 2021) or to consume dead springtails (Bilde et al., 2001). *Nebria* spp. are not considered as Collembola specialists (Baulechner et al., 2020) even if the number of individuals having consumed springtails seems to indicate a certain hunting efficiency for this prey, or at least a frequent scavenging. Preyed Diptera belonged mostly to the families Sciaridae and Chironomidae, which are among the main Diptera taxa found in agricultural landscapes (Delettre & Lagerl, 1992; Frouz, 1997). *Episyrphus balteatus* (De Geer) (Syrphidae) is also a Diptera prey often encountered. Although these results suggest a probably weak role of *N. salina* as pest regulator, and even a potential negative effect on decomposers, the predation on non-pest prey could maintain carabid populations within the field during the pest dearth periods, thereby allowing predation on pests during the following spring (Harwood & Obrichi, 2005; King et al., 2010). Thus, it is necessary to assess the diet of *N. salina* in spring, when it is the second most abundant species in oilseed rape in the ZAPVS, to know whether *N. salina* turns into a pest predator.

Calathus fuscipes on the other hand can prey on insect pupae and fruits (Larochelle, 1990). In our study, the species seems to be more oligophagous than *N. salina*. Two main prey species are preyed on, the generalist predator *A. erberi* and the anecic worm *A. longa*. As for *N. salina*, this prey spectrum could lead to reject *C. fuscipes* as potential biocontrol agent. However, the low number of individuals analysed calls for caution when concluding on these predations on one generalist predator and one decomposer. Moreover, *C. fuscipes* emerges at the end of the spring and it may have a different diet during the summer.

In conclusion, our data showed that the trapping efficiency is influenced by the trap type and the sampling session in regard to the density-activity. PCR success rate was higher only for *N. salina* specimens caught in dry pitfall traps compared to individuals caught in classic ones probably because of the combined effects of inhibitors present in the classic pitfall traps and in the digestive tract of *N. salina* due to its diet containing more plants. In addition, the ability to retrieve prey OTUs was higher for dry pitfall traps. As suggested by the accumulation curves and the beta diversity for *N. salina*, the prey OTUs composition is higher for specimens caught in dry pitfall traps.

Although frequencies of occurrence varied between the two trapping methods, the list of prey taxa detected were almost identical for individuals of the same species whether they were caught in classic or in dry pitfall traps. As Collembola were trapped in larger quantities, we can also hypothesize that the overrepresentation of Entomobryomorpha in the diet of carabids trapped in classic pitfall traps could be the result of contamination through the killing agent.

As compared to classic ones, dry pitfall traps presented the added advantage of being more focused on the target taxa and less invasive for invertebrate communities. Furthermore, the capture of live specimens presents an alternative to collect regurgitates and release the insects (Waldner & Traugott, 2012). These advantages make dry pitfalls a preferred choice, especially when repeated or intensive sampling is required. However, in this study, we cannot totally reject the hypothesis of internal trap predation. Therefore, future experiments should aim at measuring this phenomenon in dry pitfall traps.

AUTHOR CONTRIBUTIONS

Yohann Graux: Conceptualization (equal); methodology (lead); visualization (lead); writing – original draft (lead); writing – review and editing (equal). **Marina Querejeta:** Methodology (supporting); writing – review and editing (equal). **Sabrina Gaba:** Resources (equal); writing – review and editing (equal). **Vincent Bretagnolle:** Resources (equal); supervision (equal); writing – review and editing (equal). **Stéphane Boyer:** Conceptualization (equal); funding acquisition (lead); supervision (equal); writing – original draft (supporting); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in figshare at 10.6084/m9.figshare.24347140.

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