Journal of Insect Conservation (2021) 25:629–642
https://doi.org/10.1007/s10841-021-00329-4

ORIGINAL PAPER

# A novel trap design for non-lethal monitoring of dung beetles using eDNA metabarcoding

**Leandro Camila[1] · Dejean Tony[2] · Valentini Alice[2] · Jean Pauline[2] · Jay-Robert Pierre[1]**

Received: 2 March 2020 / Accepted: 4 June 2021 / Published online: 21 June 2021


## Abstract

The current biodiversity crisis calls for rapid and wide-ranging surveys to assess living organisms. However, some taxa are more elusive than others, making monitoring challenging. This is the case for soil invertebrates, but new molecular technologies such as eDNA metabarcoding could help to alleviate this problem. In this study, we evaluated the feasibility of using an eDNA approach to survey dung beetles, adapting existing monitoring methods for surveying dung fauna to enable eDNA collection in a non-destructive way. The main design idea is to capture species secretions and excretions from a serum-soaked nonwoven compress in a baited non-destructive trap. While the attractiveness of the device to dung beetles and the sampling protocol would benefit from further development, eDNA allowed the identification of more than 68% of trapped species and an identification of relative abundance match rate of 79%. The results of the study demonstrate the effectiveness of eDNA-based detection tools for the monitoring of dung beetles compared to standard surveying and identification techniques. Moreover, the adapted collecting device developed for the study could be used for similar surveys of other terrestrial invertebrates or even re-adapted. Ultimately, we hope this study encourages more non-invasive studies of insects by enabling others to utilize these emerging, non-destructive molecular techniques and therefore foster wide insect monitorings and conservation programs.

**Implications for insect conservation** Standardization and optimization of sampling protocols for inventorying and monitoring is key to unlock invertebrates' studies and conservation evaluations. Here we show how molecular tools, such as eDNA, are a promising way to gather rapidly ecological information without killing targeted populations by adapting traditional inventory tools. Newly developed NDC traps for dung beetles, inspired by CSR traps, allowed qualitative and quantitative information gathering in temperate agropastoral ecosystems opening the way to large scale eDNA monitoring to inform management and conservation schemes.

**Keywords** Biodiversity · Conservation · Soil organisms · Environmental DNA · Metabarcoding · Non-destructive sampling methods

## Introduction

The current biodiversity crisis is affecting all species, large and small (Régnier et al. 2015; Cardoso et al. 2020). Habel et al. (2019) mention several terrestrial invertebrates', such as butterflies, saproxilyc and carabid beetles, undergoing severe declining trends of their abundance and diversity in different European ecosystems. Yet surveying tiny or elusive organisms can be a difficult task (Lewis et al. 2007; Port et al. 2016), impeding conservation efforts. This is the case for invertebrates (Cardoso et al. 2011; Leandro et al. 2017): while they represent the vast majority of known species (Purvis and Hector 2000) and are suffering alarming declines (Dunn 2005; Hallmann et al. 2017), invertebrates are often not included in monitoring efforts and conservation strategies (Small 2012; Donaldson et al. 2016). The scarcity of experts and the lack of practical survey methods accentuate this deficiency (Lewis et al. 2007). This methodological issue deserves urgent attention (Hochkirch et al. 2020).

✉ Leandro Camila
camila.leandro@cefe.cnrs.fr

[1] CEFE, Univ. Paul Valéry Montpellier 3, CNRS, Univ. Montpellier, EPHE, IRD, Montpellier, France

[2] SPYGEN Savoie Technolac - Bât. Koala, 17, Rue du Lac Saint-André - BP 274, 73375 Le Bourget-du-Lac Cedex, France

In the last decade, environmental DNA (eDNA) monitoring methods have been proposed as a tool that can overcome some of the difficulties inherent to the monitoring of biodiversity (Thomsen and Willerslev 2015). This approach detects and identifies taxa from intracellular and extracellular material found in the environment (Taberlet et al. 2012), making the detection of elusive and cryptic species easier. Additionally, as it saves time and does not require taxonomical expertise, it makes large-scale and long-term monitoring schemes more feasible, particularly for non-specialists (Mächler et al. 2014; Thomsen and Willerslev 2015). Another advantage is that it solves the 'kill it to save it' paradox, as most invertebrates need to be killed for identification (New 1999; Lecq et al. 2015). This last point is more important than ever to take into account for biodiversity monitoring schemes as the 6$^{th}$ extinction is ravaging many populations across the globe (Kuussaari et al. 2009), and though entomological activities are not a major cause of insect decline at large scale, it is time to show some ethical obligations to insects (which has been poorly addressed since Lockwood 1988 call for it), especially in conservation research.

To date, DNA detection and identification techniques have largely focused on aquatic ecosystems where eDNA is made more accessible by water circulation (Rees et al. 2014; Lawson Handley 2015; Barnes and Turner 2016; Valentini et al. 2016). In terrestrial ecosystems, the flow of organic matter is more compartmentalized. As a result, the monitoring design requires adaptation in order to capture DNA traces of targeted species from the environment in a representative way and in the framework of the monitoring objective (Barnes and Turner 2016). Some authors collected eDNA from soil samples (Bienert et al. 2012; Decaëns et al. 2013) or dust to collect eDNA from their home (Madden et al. 2016). In order to survey interactions (predation, pollination…), others have sampled spider webs (Xu et al. 2015) or inflorescences (Thomsen and Sigsgaard 2019).

Dung beetles are an emblematic group of species belonging to the Scarabaeinae, Aphodiinae and Geotrupinae subfamilies. They contribute significantly to nutrient cycling, bioturbation, plant growth, seed dispersal and cattle parasite control, in tropical and temperate ecosystems (Nichols et al. 2008). In northern ecosystems, dung beetles are threatened by habitat artificialization, abandonment of traditional pastoral practices and the exposure to toxic veterinary substances (Buse et al. 2015; Verdú et al. 2018). Their functional importance in pastoral ecosystems (Losey and Vaughan 2006; Manning and Ford 2016) and their sensitivity to habitat change (McGeoch et al. 2002; Viegas et al. 2014; Tocco and Villet 2016) and to pollution (Verdú et al. 2018) make dung beetles good biological indicators (Nichols and Gardner 2011; Audino et al. 2014; Beiroz et al. 2018). With over 6,000 species known worldwide, the diversity of dung beetles is modest compared to other invertebrate groups (Simmons and Ridsdill-Smith 2011). Unfortunately, the discretion of these beetles, most of which have a small body and dark colour and divide their time between soil and dungand, mean that most species are not easy to observe or to identify. In Europe, for example, one of the most widespread species, *Onthophagus vacca* (Linnaeus, 1767), has recently been found to be a cryptic complex species thanks to research based on genetic markers (Rössner et al. 2010). These factors along with the lack of dung beetle specialists in biodiversity conservation institutions mean that these species are not considered in conservation policies (Leandro et al. 2017).

Blanckenhorn et al. (2016) worked on a metabarcoding approach to inventory dung beetles and flies from dung pads in a laboratory context: they got high sensitivity of their amplification and sequencing methods for species detection, but low correlation on species abundances and collected DNA. With these promising results, we chose to try eDNA metabarcoding, as an effective way to overcome methodological obstacles and make dung beetles more accessible to research.

Dung beetles live in a variety of habitats – pastures, forests, deserts – and have different foraging and soil-nesting behaviour; however, they all rely on dung. Although different dung beetle species may have certain trophic preferences or are attracted to dung from different origins (omnivores, ruminants, monogastric herbivores, etc.), most have a large trophic spectrum (Martín-Piera and Lobo 1996; Dormont et al. 2007; Wurmitzer et al. 2017). Dung attractiveness also partly depends on climatic conditions (Errouissi et al. 2004). Dung beetles converge towards dung by flying or crawling, as they are attracted by this resource, and then exhibit different types of behaviour to move away from it (digging under (tunnelers) or rolling and digging further away (rollers)) unless they develop in the resource (dwellers) (Doube 1990).

The typical sampling strategy for dung beetles is to use pitfall traps baited with natural dung. That could catch individuals before they move away (and often before they reach the bait). The most commonly used pitfall trap (the CSR model, described by Lobo et al. 1988 and Veiga et al. 1989) catches all three guilds of dung beetles: dwellers, tunnelers and rollers (Doube 1990). These traps can be very effective: in a study in Mediterranean areas, five CSR traps collected individuals from more than 75% of local species, representing more than 95% of the biomass of the dung beetle community (Lobo et al. 1998). Nevertheless, beetles and other species (Spiders, ground and rove beetles among others) caught by CSR traps die during sampling. For instance, a spring field campaign conducted in 2017 on 18 sites in Southern France, with five CSR traps per site, conducted us to collect more than 12,000 dung beetle individuals and

more than 2,000 non-targeted invertebrates. Such a massive impact should be if possible avoided while inventorying insects.

Therefore, this work tries to address if it is possible to adapt dung beetle monitoring tools to implement an eDNA metabarcoding approach and if a standardized and non-destructive collection device allows to validate the composition of a dung beetle community in situ. To our knowledge, this is the first field experiment with a standardize device targeting dung beetles that follows Blanckenhorn et al. (2016) work and recommendations. To test the potential of an artificial eDNA collector as a reliable tool to monitor dung beetles in the field, we (i) designed a non-destructive collecting device; (ii) checked if eDNA from dung beetles could be detected from the collection device and used to identify species; and (iii) evaluated if the amount of DNA found was related to the known community structure.

## Materials and methods

### The non-destructive collecting device

For our study, we adapted a CSR trap to create a non-lethal, non-destructive collecting device (hereafter, NDC device) designed to capture DNA from the targeted species. A standard CSR trap consists of a plastic bowl (20 cm in diameter), which is buried to its rim in the soil and contains water. A grid placed over the bowl supports the bait. Attracted by the bait, the insects slip through the grid, fall into the bowl and drown. Considering that this type of baited trap allows species to be collected in a very small volume (therefore light and user-transportable), we decided to use it as the baseline idea.

The aim was to catch the beetles, keep them alive during a determined amount of time in order to let them deposit intracellular and/or extracellular material and then be able to collect eDNA to identify and quantify the species. This required changing the CSR design and laboratory process in such a way as (i) to ensure the survival of collected individuals without permitting them to escape before leaving a trace and (ii) to avoid contamination of the collected eDNA (supporting information A).

Our NDC device (Fig. 1) was a pitfall trap made with a PVC tube 10 cm in diameter with a soft funnel at the entry (to prevent the exit of insects from the trap) and a cone-shaped bait container made of mosquito net in the top (to prevent the insects from entering the bait). At the bottom of the bait container, a sponge (3 cm in diameter, 2 mm thick) was included in order to avoid liquid from the bait (rich in microorganisms and digestive enzymes) to run into the trap. At the bottom of the trap, a sterile nonwoven compress soaked with 50 ml of commercial sterile physiological serum (Physiodose from Laboratoires Gilbert, in our case) enabled the collection of DNA from the trapped species (from secretions and excretions).

The device was designed and constructed at the Mediterranean Centre of the Environment and Biodiversity Laboratory of Excellence's (LabEx CeMEB) platform for field experiments. Before each experiment, the NDC device was decontaminated (using a 10% dilution of commercial bleach for 24 h).

### Insect detection

To compare the detection rate of the NDC device versus standard CSR traps, we conducted two field experiments on 25–26 June (session 1) and 4–5 July 2017 (session 2) on a goat farm in the scrubland north of Montpellier, France [43°48′37.0"N, 3°43′50.6"E]. The dominant vegetation was Mediterranean scrub and holm oak (*Quercus ilex* L., 1753). The temperature fluctuated between 19 and 32 °C during this period.

On both occasions, 10 CSR traps and 10 NDC devices were installed in a parcel on the site at a distance of 10 m apart and left for 24 h. According to Lobo et al. (1998), 10 CSR traps should result in the capture of beetles representing more than 85% of the local species richness and approximately 60% of the regional species pool. From field experience, 24 h was determined as the smallest amount of time required to get a picture of the dung beetle community; in this amount of time, trapped beetles were not able to escape the trap in order to get eDNA signatures in the NDC device. In each trap, we used 300 g of cow dung as bait to draw the maximum amount of the dung beetle community into the traps, as this resource is highly attractive (Wurmitzer et al. 2017).

After 24 h, all insects from the CSR traps and NDC devices were collected and stored in 95° alcohol. All specimens were counted and identified with a binocular magnifier to species level based on their morphology using the Paulian and Baraud (1982) dichotomous key and updated nomenclature (http://www.faunaeur.org). We designated this as the 'classical identification method' (CIM), which we used as a baseline to compare both the attractiveness of the two types of traps to different species and to estimate the effectiveness of the eDNA detection method (by comparing the list of species identified by CIM and by eDNA metabarcoding).

We assessed the sampling effort using species accumulation curves (the 'specaccum' function in the Vegan R package, 'exact' method, 999 permutations) (Oksanen et al. 2015). We also calculated the expected diversity and Shannon's equitability index for each type of sampling device, comparing species diversity and abundance in each device using the non-parametric Wilcoxon test (known as

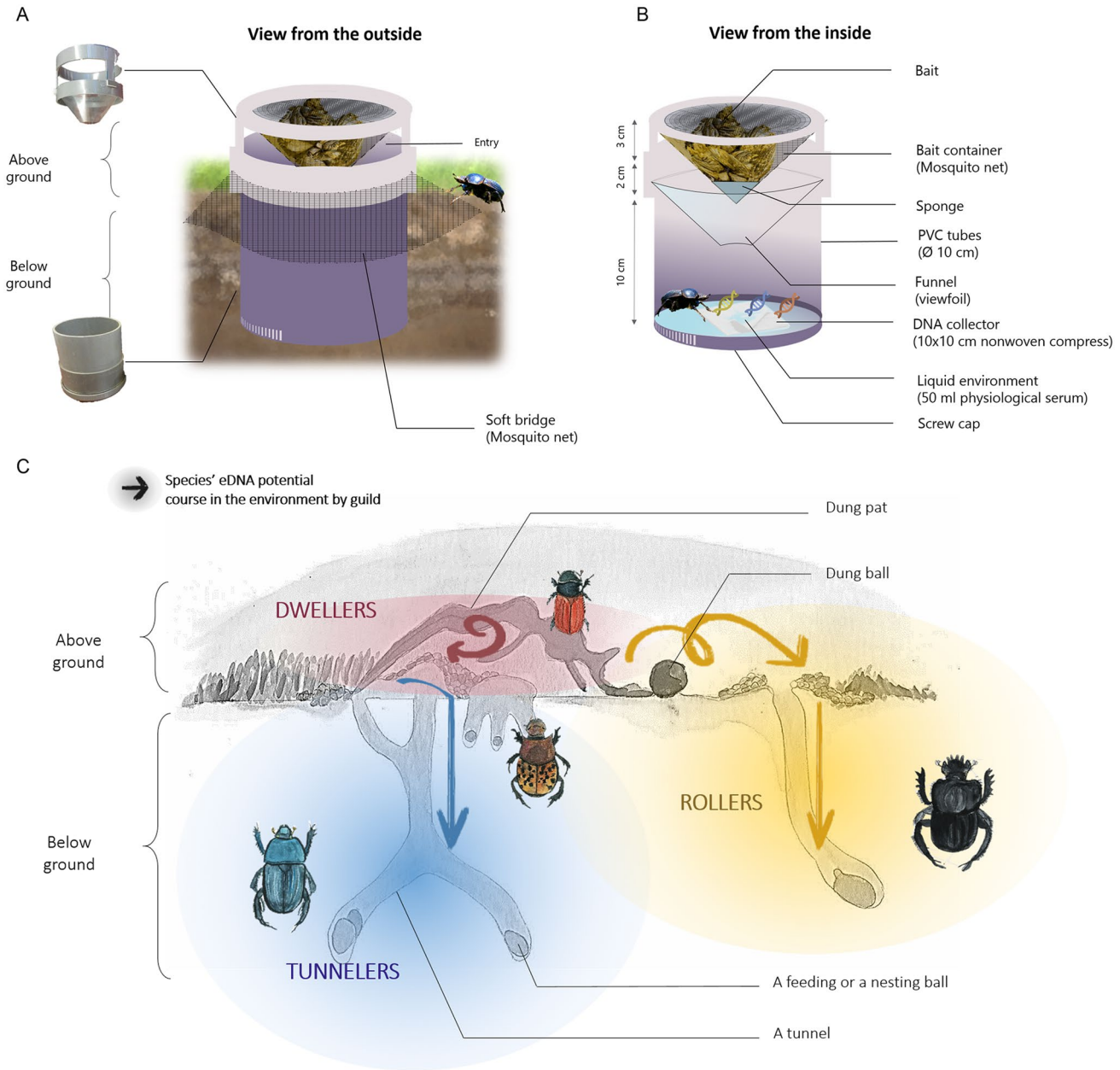


**Fig. 1** **A** The non-destructive collecting (NDC) device deployed in the field with the bait suspended above the ground and a bridge of mosquito netting to allow insects access to the trap; **B** A cutaway of the device with the bait and liquid environment to collect DNA. **C** Dung beetles demonstrate three kinds of foraging and nesting behaviour or guilds departing from the dung pad: (i) *rollers* roll a ball of dung some meters far from it, (ii) *tunnelers* live underneath the dung source and stock balls from this in tunnels that range from some cm to 1.5 m of depth, and (iii) *dwellers* live in the source of dung itself. Therefore, their DNA traces can be found in multiple locations, as the arrows show. The aim of NDC design is to get into the trap species from the different kind of guilds in one artificial environment by baiting the non-lethal trap and then to collect extracellular material (cell bristles, secretions and excretions) in order to get their DNA in a from serum-soaked nonwoven compress and proceed with eDNA metabarcoding (Illustration by CL)

'Mann–Whitney's test also). Statistical analyses were computed with R 3.4.1 (R Core Team 2017).

## Laboratory and bioinformatics analyses of eDNA

In the field, the nonwoven compresses used for eDNA collection were taken from the NDC devices with decontaminated pliers (commercial bleach at 10% for 24 h) and put in a bottle containing CL1 buffer solution (see details below).

The physiological serum was also recovered using a decontaminated funnel and added to the CL1 buffer solution. The bottles were stored in a room at 20 °C until genetic analysis.

DNA extraction was performed in a room dedicated to DNA extraction from water samples, equipped with positive air pressure, UV treatment and frequent air renewal. Before entering this extraction room, personnel changed in a connecting zone into full protective clothing comprising a disposable body suit with hood, mask, laboratory shoes, overshoes and gloves. All benches were decontaminated with 10% commercial bleach before and after each handling of a sample. The eDNA extraction was performed following a modified protocol described in Pont et al. (2018). Each bottle containing the CL1 buffer (SPYGEN, Le Bourget du Lac, France) and the nonwoven compress was agitated for 1 min on an S50 shaker (cat Ingenieurbüro™) at 800 rpm, then 15 mL of the buffer was removed with a sterile pipette and added to a 50-mL tube containing 33 mL of ethanol and 1.5 mL of 3 M sodium acetate. The tubes were stored for at least one night at -20 °C. They were then centrifuged at 15,000×*g* for 15 min at 6 °C, and the supernatants were discarded. After this step, 720 µL of ATL buffer from the DNeasy Blood and Tissue Extraction Kit (Qiagen, Hilden, Germany) was added. The tubes were then vortexed, and the supernatants were transferred to 2-mL tubes containing 20 µL of Proteinase K. The tubes were finally incubated at 56 °C for two hours. Subsequently, DNA extraction was performed using NucleoSpin® Soil (MACHEREY–NAGEL GmbH and Co., Düren, Germany) kit manual starting from step 6 and following the manufacturer's instructions. The elution was performed by adding 100 µL of SE buffer twice. One negative extraction control was also performed. After the DNA extraction, the samples were tested for inhibition by qPCR following the protocol in Biggs et al. (2015). Five samples were considered inhibited and the DNA was diluted fivefold before amplification.

To identify the beetle species, we used the primers couple ins_F/ins_R (Elbrecht et al. 2016) to amplify a fragment of ~157 bp of the mitochondrial 16S gene. The DNA amplifications were done in a 25 µL solution that included 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA), 10 mM of Tris–HCl, 50 mM of KCl, 2.5 mM of MgCl2, 0.2 mM of each dNTP, 0.2 µM of each primer, 0.2 µg/µL of bovine serum albumin (BSA, Roche Diagnostic, Basel, Switzerland) and 3 µL of DNA template. The primers were 5'-labeled with an eight-nucleotide tag unique to each sample (with at least three differences between any pair of tags), allowing each sequence to be assigned to the corresponding sample during the sequence analysis. The forward and reverse primer tags were identical. The PCR mixture was denatured at 95 °C for 10 min, followed by 45 cycles of 30 s at 95 °C, 30 s at 56 °C and 1 min at 72 °C and a final elongation step at 72 °C for 7 min in a room dedicated to amplified DNA with negative air pressure and physical separation from the DNA extraction rooms (with positive air pressure). Twelve PCR replicates were performed per sample. One negative extraction control and one PCR control were amplified (12 replicates as well) and sequenced in parallel. The purified PCR products were pooled in equal volumes to achieve an expected sequencing depth of 100,000 reads per sample. Library preparation and sequencing were performed at Fasteris facilities (Geneva, Switzerland). The library was prepared using the Metafast protocol (https://www.fasteris.com/metafast). The library was sequenced using Illumina MiSeq (2 × 150 bp) (Illumina, San Diego, CA, USA) and the MiSeq Reagent Kit v3 (Illumina, San Diego, CA) following the manufacturer's instructions.

## Reference database construction and bioinformatics analyses of eDNA

As a control, DNA was extracted from 10-mg tissue samples from a leg of the 18 species of Geotrupinae, Scarabaeinae and Aphodiinae (*Ammoecius elevatus, Aphodius fimetarius, Caccobius schreberi, Colobopterus erraticus, Copris hispanus, Euoniticellus fulvus, Onthophagus coenobita, Onthophagus furcatus, Onthophagus grossepunctatus, Onthophagus joannae, Onthophagus lemur, Onthophagus maki, Onthophagus ovatus, Onthophagus taurus, Onthophagus vacca, Onthophagus verticicornis, Otophorus haemorrhoidalis*, *Sisyphus schaefferi*) trapped with the NDC device, using DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) in a room dedicated to DNA extraction and following the manufacturer's instructions. DNA amplifications and sequencing were performed as described above. The purified PCR products were pooled in equal volumes to achieve an expected sequencing depth of 50,000 reads per sample.

The sequence reads for the eDNA samples and the reference database were analysed using the programs in the OBITools package (http://metabarcoding.org/obitools; Boyer et al. 2016) following the protocol described in Valentini et al. (2016). The forward and reverse reads were assembled using the *illuminapairedend* program with a minimum score of 40 and retrieving only joined sequences. The reads were then assigned to each sample using the *ngsfilter* program. A separate dataset was created for each sample by splitting the original dataset into several files using *obisplit*. After this step, each sample was analysed individually before merging the taxon list for the final ecological analysis. Strictly identical sequences were clustered together using *obiuniq*. Sequences shorter than 20 bp (that may correspond to primer

dimer), or with occurrences lower than 10 per sample (that may correspond to PCR errors), were excluded using the *obigrep* program. The *obiclean* program was then run to assign the status of 'head' (most common sequence among all sequences that can be linked with a single indel or substitution), 'singleton' (no other variant with a single difference in the relevant PCR product) or 'internal' (all other sequences not being 'head' or 'singleton', i.e. corresponding to amplification/sequencing errors) to each sequence within a PCR product. All sequences labelled 'internal', that corresponded most likely to PCR substitutions and thus errors, were discarded. Taxonomic assignment of the molecular operational taxonomic units (MOTUs) was performed using the program *ecotag* and the sequences extracted from ENA Release 127 (standard sequences) of the European Bioinformatics Institute's EMBL database using the ECOPCR program (Bellemain et al. 2010; Ficetola et al. 2010) and with the local reference database built for this study. Only MOTUs showing a similarity higher than 98% with either the local or the EMBL reference databases were retrieved for the analysis (Meyer et al. 2020). Finally, we discarded all MOTUs with an occurrence frequency below 0.001 per library.

## Results

### CSR trap vs NDC device capture efficiency

In total, 3,884 individuals from 21 species of dung beetles were trapped during the experiment. As in Mediterranean habitats the drought season sets in as the weeks go by, insect activity in session 2 was expected to be lower. Therefore, we decided to not pool data for the analysis.

Using the classical identification method (CIM), the NDC device was less attractive to dung beetles than the standard CSR trap (Table 1). In session 1, the 10 CSR traps caught 17 species, while the same number of NDC devices caught 15 species. In session 2, the total captures were 19 and 11 respectively. Species accumulation curves and Chao's estimator (Fig. 2A and B) also revealed differences in the number of species caught by each type of trap, particularly for session 2: for the 10 CSR traps, total estimated richness was 19.45 (±0.95), while for NDC devices it was 11.9 (±1.71). In session 1, four species were trapped only with the CSR traps, while two species were exclusively observed in NDC devices; during session 2, the CSR traps attracted nine species never observed in the NDC devices, while only one species was exclusively trapped in a NDC device. For instance, Geotrupinae, which represented 0.3% of captures in CSR traps, were never caught in NDC devices (Table 2). Of Scarabaeinae and Aphodiinae, three species were never caught in NDC devices, yet together they only represented 0.4% of CSR captures. Four other species were trapped by NDC devices only but not detected by eDNA identification method, although these represented 2.2% of the total captures (by CSR and NDC devices).

Despite the lesser attractiveness for rare species, the NDC device showed a higher equitability value (Wilcoxon test p-value < 0.01 for session 1; p-value = 0.06 for session 2) of the collected diversity and a statistically higher number of species/number of specimens' ratio (Wilcoxon test p-value < 0.001 for both sessions) (Fig. 2C and D). Moreover, the abundance correlation of shared species between sampling devices was high for both sessions (Spearman R = 0.826 p-value < 0.001 for S1; Spearman R = 0.845 p-value < 0.001 for S2).

### eDNA detection efficiency

In session 1, 69.5% and in session 2, 68.8% of the species captured in NDC devices and identified by CIM were also detected through eDNA (Table 2). The species detected by eDNA always represented the majority of the CSR captures in terms of abundance: 96.6% (belonging to 10 species) during the first session, and 95.3% (belonging to 7 species) during the second session.

Moreover, in some NDC devices from session 1, three species were detected by eDNA yet not found in the corresponding NDC device. Nevertheless, the three species concerned were captured and detected in other devices during their corresponding field session. Furthermore, NDC devices allowed the detection of several other beetles. Carabidae (1 species), Dermestidae (1 species), Hydrophilidae (1 species) and Staphylinidae (3 species) were detected in 12 out of the 20 devices. These taxa were detected by comparing the DNA collected to the sequences available on public reference sequence databases. Likewise, 11 species

**Table 1** Summary of principal differences in results between the NDC device and the CSR trap (S1: session 1, S2: session 2, W: result of the Wilcoxon test)

| | | CSR | NDC | P-VALUE |
|---|---|---|---|---|
| Mean species richness per trap | S1 | 9.7 ± 2.58 | 4.6 ± 2.50 | 0.001 (W 92.5) |
| | S2 | 9.7 ± 2.79 | 5.9 ± 1.19 | 0.002 (W 90.5) |
| Mean abundance per trap | S1 | 175.2 ± 49.62 | 33.8 ± 16.46 | 0.0001 (W 100) |
| | S2 | 134 ± 48.62 | 43.3 ± 14.87 | 0.0002 (W 99.0) |

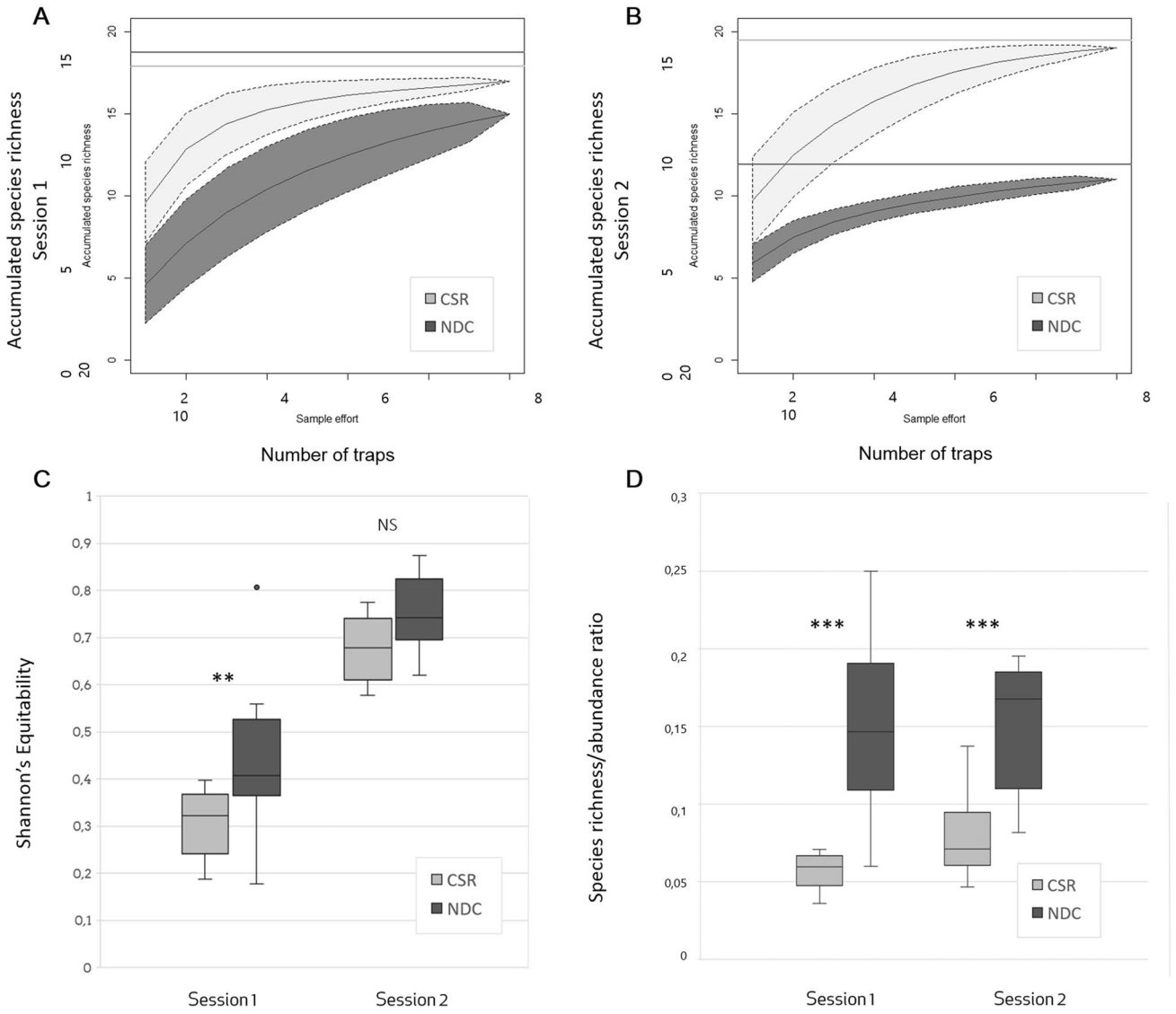


**Fig. 2** Species diversity and equitability for each collection device. **A** and **B** show species accumulation curves with envelopes ('exact' method, 999 permutations) and Chao's diversity estimation (horizontal line) for each device for session 1 and 2 respectively. **C** shows the Shannon's equitability (evenness) index and **D** shows the species richness/abundance ratio for both types of trap in each session; significant differences (Wilcoxon test) are shown in the graphic (Signif. codes: 0.01 '**', 0.001 '***' and NS if > 0.05)

from the Diptera order were found. These insects were observed but not identified by CIM (the largest species, one specimen of Carabus sp., was released during the collection of the devices).

The correlation between the number of individuals caught in NDC devices and the amount of eDNA detected (number of reads) was significant (Spearman's $R = 0.461$ p-value $< 0.001$; Fig. 3A). Furthermore, the fidelity of ranking between the number of specimens trapped in the device and the amount of eDNA detected was perfect in 47.8% of cases (Fig. 3B, dark green) and high (with ± one rank of difference) in 31.3% of cases (Fig. 3B, light green). Even the rarest species might be detected and their relative abundance estimated by eDNA: this was the case for *Copris lunaris* (large species), *Colobopterus erraticus* (medium species), and *Onthophagus joannae* (a small species), each as single individuals found in one trap from session 1.

## Discussion

### The NDC device

Dung beetles are a somewhat paradoxical study case: while they are among relatively well known insects,

**Table 2** Comparison between collecting methods (CSR vs NDC) and identification methods (CIM vs eDNA, for samples collected by NDC) by field session

| Species by subfamily | Species traits | | Session 1 | | | Session 2 | | |
|---|---|---|---|---|---|---|---|---|
| | mean body size (in mm) | Guild | CSR trap | NDC trap | | CSR trap | NDC trap | |
| | | | CIM | CIM | eDNA Identification method | CIM | CIM | eDNA Identification method |
| Geotrupinae subfamily | | | | | | | | |
| *Geotrupes mutator* (Marsham, 1802) | *19* | *P* | 0 | 0 | 0 | 1 | 0 | 0 |
| *Geotrupes spiniger* Marsham, 1802 | *21* | *P* | 3 | 0 | 0 | 5 | 0 | 0 |
| *Sericotrupes niger* (Marsham, 1802) | *19* | *P* | 0 | 0 | 0 | 1 | 0 | 0 |
| Scarabaeinae subfamily | | | | | | | | |
| ***Caccobius schreberi* (Linnaeus, 1767)** | *5.5* | *P* | 51 | 7 | 14 | 198 | 23 | 853 |
| ***Copris hispanus hispanus* (Linnaeus, 1764)** | *25* | *P* | 0 | 1 | 52 | 0 | 0 | 0 |
| ***Euoniticellus fulvus* (Goeze, 1777)** | *9* | *P* | 102 | 23 | 219,387 | 466 | 108 | 385,757 |
| *Euonthophagus amyntas* (Olivier, 1789) | *9.5* | *P* | 7 | 0 | 0 | 2 | 0 | 0 |
| ***Onthophagus coenobita* (Herbst, 1783)** | *8* | *P* | 5 | 3 | 35 | 4 | 0 | 0 |
| *Onthophagus furcatus* (Fabricius, 1781) | *4.3* | *P* | 1 | 0 | 0 | 3 | 0 | 0 |
| *Onthophagus grossepunctatus* Reitter, 1905 | *4.5* | *P* | 12 | 4 | 0 | 11 | 78 | 0 |
| *Onthophagus joannae* Goljan, 1953 | *5* | *P* | 11 | 1 | 453 | 16 | 0 | 5294 |
| *Onthophagus lemur* (Fabricius, 1782) | *7* | *P* | 10 | 2 | 0 | 2 | 0 | 0 |
| ***Onthophagus maki* (Illiger, 1803)** | *5.5* | *P* | 0 | 1 | 237 | 2 | 5 | 14 |
| ***Onthophagus taurus* (Schreber, 1759)** | *8.5* | *P* | 19 | 7 | 2569 | 20 | 8 | 13,733 |
| *Onthophagus vacca* (Linnaeus, 1767) | *10* | *P* | 4 | 1 | 0 | 18 | 6 | 0 |
| ***Onthophagus verticicornis* (Laicharting, 1781)** | *7.5* | *P* | 23 | 3 | 28 | 2 | 1 | 0 |
| ***Sisyphus schaefferi* (Linnaeus, 1758)** | *9.3* | *T* | 1470 | 276 | 51,888 | 476 | 75 | 2865 |
| Aphodiinae subfamily | | | | | | | | |
| *Ammoecius elevatus* [7] | *7* | *E* | 0 | 0 | 0 | 0 | 1 | 0 |
| *Aphodius fimetarius* (Linnaeus, 1758) | *6.5* | *E* | 5 | 2 | 0 | 4 | 2 | 0 |
| ***Colobopterus erraticus* (Linnaeus, 1758)** | *7.5* | *P* | 11 | 1 | 19,999 | 6 | 0 | 0 |
| ***Otophorus haemorrhoidalis* (Linnaeus, 1758)** | *4.5* | *E* | 17 | 6 | 48,920 | 56 | 192 | 242,854 |
| *Teuchestes fossor* (Linnaeus, 1758) | *10* | *E* | 1 | 0 | 0 | 0 | 0 | 0 |

We specified the number of caught individuals by trap (abundances) and by species, and for NDC devices we also included the number of reads (DNA abundance). Species, for which we specified their mean body length and guild (E: Encoprid, P: Paracoprid and T: Telecoprid) based on literature review, were highlighted in bold if caught by the NDC device and detected through eDNA

provide essential ecosystem services (Losey and Vaughan 2006), and have been identified as good potential indicators regarding habitat conservation (Nichols and Gardner 2011), their study is restricted to a very small circle of specialists, despite the fact that no research drawbacks have been revealed about them. This paradox is largely due to the difficulty of accurately identifying most of these species without killing them and using dissecting techniques and binocular magnifiers. CSR traps have proven to be a very efficient tool to sample dung beetles whatever the habitat (Veiga et al. 1989; Lobo et al. 1998; Larsen and Forsyth 2005); however, these traps deliver a large number of (dead) specimens, requiring many hours of morphological identification and specialized optical material, which makes extensive monitoring use expensive and time-consuming.

To attempt to address these concerns, we designed a non-destructive DNA collecting (NDC) device by adapting a CSR trap in order to collect eDNA from trapped dung beetles without killing them. All materials and possible settings were tested before in order to ensure this (more detail on the supporting information A).

The device proved to be efficient: the comparison with CSR results showed that the bulk of the local assemblage was properly characterized and, moreover, from the high equitability and the high number of species/number of specimens' ratios we might deduce that the NDC device was not prone to over-catch the dominant species, which is a failing of numerous trapping methods. The NDC device makes the

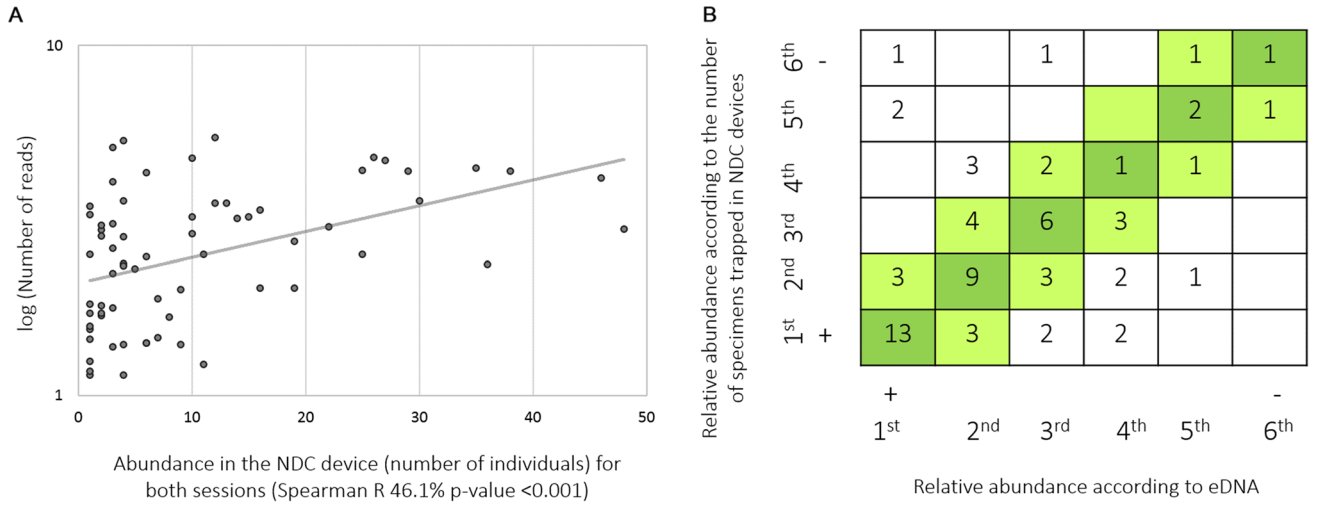


**Fig. 3** **A** Relationships between the abundance of individuals for each species in each NDC trap and the number of reads (log scale) of dung beetle species, both sessions combined. **B** Ranking of species according to their relative abundance in the NDC device and to the relative abundance of eDNA measured in the trap, both sessions combined. Dark green: same ranking position (i.e. perfect detection); light green: one rank of difference (i.e. high detection). (Color figure online)

sampling of dung beetles feasible as it is compact, light, made of easily available materials and requires no more than 50 mL of water (while approximately 3 L are needed for a CSR trap). Therefore, it is easy to transport, to construct and to use in the field by inexperienced operators– a key criterion for developing molecular survey methods (Taylor et al. 2017). The cost of the device and all needed material would be 10€ each, close to a CRS trap; the analysis of the soaked nonwoven compress by eDNA metabarcoding is ~ 200 € and should be cheaper in the decades to come, no matter how many species are in the trap, whether a whole day of identification activity by an experienced entomologist costs around 300 € (supporting information A).

The study was carried out in the Mediterranean region of southern France, an area where dung beetle fauna is highly diversified (Lumaret and Lobo 1996; Dortel et al. 2013). Lobo et al. (1998) put forward that, in this region, the use of 2–5 CSR traps may be sufficient for ecological studies dealing with the composition and structure of assemblages. Our results seem to indicate that approximately 50% more NDC devices may be needed to achieve the same goal, which is a feasible, and within everyone's pocket. In tropical or savannah contexts, we would recommend a calibration of the needed number of NDC devices in order to get the whole community before getting into eDNA studies.

Nevertheless, it could be useful to improve the attractiveness of the device as not all species were captured by the NDC device. If, on the one hand, it seems risky to enlarge the diameter of the device because the confining of insects determines the concentration of eDNA in the samples, on the other hand it may be possible to expose the bait even further, to increase its size or to consider a mix of baits (Larsen and Forsyth 2005; Spector 2006).

Some flaws of our experiment could have come from the survey design. To compare the attractiveness of NDC devices and CSR traps, 20 pitfalls were alternatively spaced at a distance of 10 m apart (40*30 m rectangle). With such a protocol, NDC devices and CSR traps highly competed for beetles (Larsen and Forsyth 2005). Moreover, the diameter of the bowl that constituted the CSR trap was twice as large as the tube that composed the NDC device. This 300 $cm^2$ open surface with 3L of water might modify the microenvironment and catch the beetles much before they have reached the bait. This water-induced bias should have been especially high during the second sampling with dryer climatic conditions. All these parameters could explain the lower efficiency of NDC devices to catch dung beetles. During a standard sampling, when interferences between the two kinds of pitfalls do not appear, a higher efficiency of NDC device should be expected.

But there are also ways we may be able to enhance our survey design and adjust it to local contexts and problems, and thus be able to more accurately study dung beetle communities (Kéry et al. 2008). The more obvious could be to sample in the absence of the livestock to avoid the competition with natural pads (Lobo et al. 1998). In different habitats, such as savannahs and topical forests, it could be convenient to also adapt the bait (Bogoni and Hernandez 2014; Correa et al. 2016); in tropical forests, take into account the vertical stratification of dung beetles (arboreal species that have a perching behaviour) (Noriega et al. 2020) could also be a possible adjustment: for instance, the NDC device

could be lifted, and a "plateau" around the entrance could be added.

A trickier issue would be to reconsider the time span of the survey on the field; indeed, leave the NDC device from 24 to 48 h or even 72 h, in order to increase attractiveness and the amount of extracellular DNA in the serum could also be a research track, nevertheless we want to draw attention to the issue of DNA degradation due to the exposure to UVs or microorganisms that could develop in the serum. So the best idea so far, in our eyes, would be to expose the bait in a better way and try to maintain it as fresh as in the CSR traps.

## eDNA detection and possibilities

As found in a study by Thomsen et al. (2012), secretions and excretions were believed to be the predominant sources of eDNA in the device. Both to avoid the overrepresentation of insects that arrived first and to ensure individual survival, it was decided to leave the traps in place for only 24 h (CSR traps are generally left in position for several days). The fact that there was a very good balance between the relative abundance based on the number of reads and the number of trapped specimens seems to indicate this solution addressed this concern. The good correlation between the quantity of DNA and the number of specimens also shows that the differences in body mass between species did not induce a significant bias in DNA sampling. Considering the large variability in body size within this group of beetles (e.g. the smallest individual was 4 mm long while the biggest was 21 mm in our study (Table 2)) (Lobo 1993), this bias was a matter of great concern (Takahara et al. 2012; Blanckenhorn et al. 2016).

We reached 69% qualitative identification rate and a 79% of good quantitative estimation rate (the sum of good and perfect abundance rank matching, Fig. 3b), therefore the efficiency of the NDC device is very similar to that of other non-visual invertebrate monitoring techniques (Mankin et al. 2011; Potamitis and Schäfer 2014). By comparing our results with those from Blanckenhorn et al. (2016), who used a metabarcoding approach with CO1 primers in a laboratory study, one can consider that our method performed qualitatively well, by achieving to depict of the bulk community on in situ conditions, but showed a quantitatively lower performance, with a weaker positive correlation (~46 in our case). Nevertheless, such a correlation was also found in studies comparing species abundances retrieved from traditional methods to genetic approaches as metabarcoding (Watts et al. 2019). Nevertheless, if we look at the quality of quantitative information through the lenses of the ranking of species (DNA abundance vs individual abundance) our method does indicate the possibility to get relative abundances from the surveyed community.

As pointed out in the results, while NDC devices did not catch all dung beetle species, they allowed the detection of several other beetles and even the detection of some fly species from the *Megaselia* and *Sepsis* genera. The use of pitfall traps is a standard way to sample ground-dwelling beetles, as they regularly fall in these traps (Ahmed and Petrovskii 2019). Yet the identification of these species is a difficult task: to give just one example, there are approximately 2,000 different rove beetle species in Europe (de Jong et al. 2014). Consequently, the study of dung insect assemblages is generally restricted to Scarabaeidae and Geotrupidae. An eDNA-based sampling method offers the possibility of identifying a large variety of taxa in the field, opening the way to a more complete study of the micro-ecosystem of dung (Hanski 1991; Blanckenhorn et al. 2016). Of ground-dwelling beetles, many species of rove beetles (Staphylinidae), ground beetles (Carabidae) and histerids (Histeridae) are predators that play a decisive role in the regulation of other dung-living organisms, and which strongly depend on the dynamics of these preys (Sheppard and Harwood 2005; Cristescu 2014). A further challenge will be to test the capacity of NDC devices to attract such species (i.e. testing different kind of baits and different materials for the fennel) and to accurately estimate the abundance of these non-coprophagous beetles, to get a broader picture of coprophilous communities.

Scientists have signalled that a current mass extinction is underway, in the context of which insects are experiencing a dramatic decline in populations and diversity (Dunn 2005; Hallmann et al. 2017; Cardoso et al. 2020). Threats to dung beetles include habitat destruction, the intensification of agricultural practices, and the exposure to toxic veterinary substances used to treat livestock, the residues of which are dispersed in dung (Buse et al. 2015; Verdú et al. 2018). In Europe, particularly Mediterranean localities, urban development of the coastal zones for mass tourism in the past 70 years and forest spread after traditional livestock raising abandonment have been pointed as major causes of the disappearance of many roller populations (Lobo 2001; Carpaneto et al. 2007), which are particularly endangered.

Emerging technologies in genomics represent challenges but also significant opportunities for monitoring, and thus for conservation (Pimm et al. 2015; Taylor and Gemmell 2016). In order to propose solutions to stem the massive decline in biodiversity, we need to be able to measure populations effectively, which relies on developing systematic, efficient – ideally non-invasive – monitoring methods. eDNA (meta)barcoding sampling methods are one promising possibility. While this study is a first step and merits further development, it shows how standard techniques can be adapted to collect DNA in order to apply a (meta)barcoding approach. Moreover, in some cases, when barcode libraries are incomplete or when the taxonomy is still debated, eDNA

metabarcoding approach developed at a supra taxonomic level (e.g. families or tribes) could supply the temporary lack of information. Such a two-step procedure shows the potential of using genetic tools to investigate the complexity of the biodiversity structure.

Insects, and more generally invertebrates, are critical to the functioning and resilience of ecosystems and must not be neglected in conservation efforts (Leather et al. 2008; Rees et al. 2014; Samways et al. 2020). They should be a priority in the development of new genetic surveying methods and equally take into account in ecosystem protection strategies.

## Conclusion

We developed a non-destructive trap, which served as an artificial eDNA collector to monitor dung beetles in the field. Our field experiments showed the value of eDNA for detecting the presence/absence and relative abundance of dung beetle species through the developed device. While both the sampling device and the design of the survey could be enhanced to further improve results (species attraction and distance between traps), the findings prove the efficacy of the eDNA metabarcoding approach. They indicate that non-destructive DNA collecting devices could be used in a systematic way in monitoring schemes to gather good quality data (Bickford et al. 2007; Baird and Hajibabaei 2012). As they are non-invasive and the identification process much less labour-intensive than using standard traps, these methods could also stimulate further ecological research on dung related fauna, increasing the number of invertebrate surveys, leading to more collected data and, ultimately, triggering conservation measures for currently overlooked species.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10841-021-00329-4.

**Acknowledgements** We would like to acknowledge D. Degueldre for helping during the conception and construction of the NDC device. We would also like to thank N. Foulquier, who allowed us to use his farm for the experiments. We are grateful to J. Bonfanti and S. Savagner for their field assistance and SPYGEN staff for their assistance in the eDNA laboratory. Finally, we would like to thank the reviewers, whose suggestions improved this manuscript.

## Declarations

**Conflict of interest** The Authors declares that there is no conflict of interest.

**Research involving human and animal participants** None of the collected species is protected in France and/or the European Union; hence, captures do not need preliminary authorization. All specimens are stored, labelled and added to the laboratory collection. Data will be published and available thus the ScaraB'Obs database (https://data.oreme.org/entomo/home).

**Informed consent** All authors agreed with the content and that all gave explicit consent to submit and that they obtained consent from the responsible authorities at the institute/organization where the work has been carried.

## References

Ahmed DA, Petrovskii SV (2019) Analysing the impact of trap shape and movement behaviour of ground-dwelling arthropods on trap efficiency. Methods Ecol Evol 10(8):1246–1264. https://doi.org/10.1111/2041-210X.13207

Audino LD, Louzada J, Comita L (2014) Dung beetles as indicators of tropical forest restoration success: Is it possible to recover species and functional diversity? Biol Conserv 169:248–257. https://doi.org/10.1016/j.biocon.2013.11.023

Baird DJ, Hajibabaei M (2012) Biomonitoring 2.0: a new paradigm in ecosystem assessment made possible by next-generation DNA sequencing. Mol Ecol 21(8):2039–2044. https://doi.org/10.1111/j.1365-294X.2012.05519.x

Barnes MA, Turner CR (2016) The ecology of environmental DNA and implications for conservation genetics. Conserv Genet 17(1):1–17. https://doi.org/10.1007/s10592-015-0775-4

Beiroz W, Sayer E, Slade EM, Audino L, Braga RF, Louzada J, Barlow J (2018) Spatial and temporal shifts in functional and taxonomic diversity of dung beetles in a human-modified tropical forest landscape. Ecol Indicat 95:518–526. https://doi.org/10.1016/j.ecolind.2018.07.062

Bellemain E, Carlsen T, Brochmann C, Coissac E, Taberlet P, Kauserud H (2010) ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. BMC Microbiol 10:189. https://doi.org/10.1186/1471-2180-10-189

Bickford D, Lohman DJ, Sodhi NS et al (2007) Cryptic species as a window on diversity and conservation. Trends Ecol Evol 22(3):148–155. https://doi.org/10.1016/j.tree.2006.11.004

Bienert F, De Danieli S, Miquel C, Coissac E, Poillot C, Brun JJ, Taberlet P (2012) Tracking earthworm communities from soil DNA. Mol Ecol 21(8):2017–2030. https://doi.org/10.1111/j.1365-294X.2011.05407.x

Biggs J, Ewald N, Valentini A, Gaboriaud C, Dejean T, Griffiths RA, Foster J, Wilkinson JW, Arnell A, Brotherton P, Williams P, Dunn F (2015) Using eDNA to develop a national citizen science-based monitoring program for the great crested newt (*Triturus cristatus*). Biol Conserv 183:19–28. https://doi.org/10.1016/j.biocon.2014.11.029

Blanckenhorn WU, Rohner P, Bernasconi MV, Haugstetter J, Buser A (2016) Is qualitative and quantitative metabarcoding of dung fauna biodiversity feasible? Environ Toxicol Chem 35(8):1970–1977. https://doi.org/10.1002/etc.3275

Bogoni JA, Hernández MI (2014) Attractiveness of native mammal's feces of different trophic guilds to dung beetles (Coleoptera: Scarabaeinae). J Insect Sci. https://doi.org/10.1093/jisesa/ieu161

Boyer F, Mercier C, Bonin A, Le Bras Y, Taberlet P, Coissac E (2016) Obitools: a unix-inspired software package for DNA metabarcoding. Mol Ecol Resour 16:176–182. https://doi.org/10.1111/1755-0998.12428

Buse J, Šlachta M, Sladecek FX, Pung M, Wagner T, Entling MH (2015) Relative importance of pasture size and grazing continuity for the long-term conservation of European dung beetles. Biol Conserv 187:112–119. https://doi.org/10.1016/j.biocon.2015.04.011

Cardoso P, Erwin TL, Borges PAV, New TR (2011) The seven impediments in invertebrate conservation and how to overcome them.

Biol Conserv 144(11):2647–2655. https://doi.org/10.1016/j.biocon.2011.07.024
Cardoso P, Barton PS, Birkhofer K, Chichorro F, Deacon C, Fartmann T, Fukushima CS, Gaigher R, Habel JC, Hallmann CA, Hill MJ, Hochkirch A, Kwak ML, Mammola S, Ari Noriega J, Orfinger AB, Pedraza F, Pryke JS, Roque FO, Settele J, Simaika JP, Stork NE, Suhling F, Vorster C, Samways MJ (2020) Scientists' warning to humanity on insect extinctions. Biol Conserv 242:108426. https://doi.org/10.1016/j.biocon.2020.108426
Carpaneto GM, Mazziotta A, Valerio L (2007) Inferring species decline from collection records: roller dung beetles in Italy (Coleoptera, Scarabaeidae). Divers Distrib 13(6):903–919. https://doi.org/10.1111/j.1472-4642.2007.00397.x
Correa CM, Puker A, Korasaki V, Ferreira KR, Abot AR (2016) Attractiveness of baits to dung beetles in Brazilian savanna and exotic pasturelands. Entomol Sci 19(2):112–123. https://doi.org/10.1111/ens.12169
Cristescu ME (2014) From barcoding single individuals to metabarcoding biological communities: towards an integrative approach to the study of global biodiversity. Trends Ecol Evol 29(10):566–571. https://doi.org/10.1016/j.tree.2014.08.001
de Jong Y, Verbeek M, Michelsen V, Bjørn P, Los W, Steeman F, Bailly N, Basire C, Chylarecki P, Stloukal E, Hagedorn G, Wetzel F, Glöckler F, Kroupa A, Korb G, Hoffmann A, Häuser C, Kohlbecker A, Müller A, Güntsch A, Stoev P, Penev L (2014) Fauna Europaea–all European animal species on the web. Biodivers Data J 2:e4034
Decaëns T, Porco D, Rougerie R, Brown GG, James SW (2013) Potential of DNA barcoding for earthworm research in taxonomy and ecology. Appl Soil Ecol 65:35–42
Donaldson MR, Burnett NJ, Braun DC, Suski CD, Hinch SG, Cooke SJ, Kerr JT (2016) Taxonomic bias and international biodiversity conservation research. Facets 1:105–113. https://doi.org/10.1139/facets-2016-0011
Dormont L, Rapior S, McKey DB, Lumaret JP (2007) Influence of dung volatiles on the process of resource selection by coprophagous beetles. Chemoecology 17(1):23–30. https://doi.org/10.1007/s00049-006-0355-7
Dortel E, Thuiller W, Lobo JM, Bohbot H, Lumaret JP, Jay-Robert P (2013) Potential effects of climate change on the distribution of Scarabaeidae dung beetles in western Europe. J Insect Conserv 17:1059–1070. https://doi.org/10.1007/s10841-013-9590-8
Doube BM (1990) A functional classification for analysis of the structure of dung beetle assemblages. Ecol Entomol 15(4):371–383. https://doi.org/10.1111/j.1365-2311.1990.tb00820.x
Dunn RR (2005) Modern insect extinctions, the neglected majority. Conserv Biol 19(4):1030–1036. https://doi.org/10.1111/j.1523-1739.2005.00078.x
Elbrecht V, Taberlet P, Dejean T et al (2016) Testing the potential of a ribosomal 16S marker for DNA metabarcoding of insects. PeerJ 4:e1966. https://doi.org/10.7717/peerj.1966
Errouissi F, Haloti S, Jay-Robert P, Janati-Idrissi A, Lumaret JP (2004) Effects of the attractiveness for dung beetles of dung pat origin and size along a climatic gradient. Environ Entomol 33(1):45–53. https://doi.org/10.1603/0046-225X-33.1.45
Ficetola GF, Coissac E, Zundel S, Riaz T, Shehzad W, Bessière J, Taberlet P, Pompanon F (2010) An In silico approach for the evaluation of DNA barcodes. BMC Genomics 11:434. https://doi.org/10.1186/1471-2164-11-434
Habel JC, Samways MJ, Schmitt T (2019) Mitigating the precipitous decline of terrestrial European insects: requirements for a new strategy. Biodiv Conserv 28:1343–1360. https://doi.org/10.1007/s10531-019-01741-8
Hallmann CA, Sorg M, Jongejans E, Siepel H, Hofland N, Schwan H, De Kroon H (2017) More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS ONE. https://doi.org/10.1371/journal.pone.0185809
Hanski I (1991) The dung insect community. In: Hanski I, Cambefort Y (eds) Dung beetle ecology. Princeton University Press, Princeton, pp 5–21
Hochkirch A, Samway MJ, Gerlach J, Böhm M, Williams P, Cardoso P et al (2020) A strategy for the next decade to address data deficiency in neglected biodiversity. Conserv Biol 35(2):502–509. https://doi.org/10.1111/cobi.13589
Kéry M, Royle JA (2008) Schmid HImportance of sampling design and analysis in animal population studies: a comment on Sergio et al. J Appl Ecol 45(3):981–986. https://doi.org/10.1111/j.1365-2664.2007.01421.x
Kuussaari M, Bommarco R, Heikkinen RK, Helm A, Krauss J, Lindborg R et al (2009) Extinction debt: a challenge for biodiversity conservation. Trends Ecol Evol 24(10):564–571. https://doi.org/10.1016/j.tree.2009.04.011
Larsen TH, Forsyth A (2005) Trap spacing and transect design for dung beetle biodiversity studies. Biotropica 37(2):322–325. https://doi.org/10.1111/j.1744-7429.2005.00042.x
Lawson Handley L (2015) How will the "molecular revolution" contribute to biological recording? Biol J Lin Soc 115(3):750–766. https://doi.org/10.1111/bij.12516
Leandro C, Jay-Robert P, Vergnes A (2017) Bias and perspectives in insect conservation: a European scale analysis. Biol Conserv 215:213–224. https://doi.org/10.1016/j.biocon.2017.07.033
Leather SR, Basset Y, Hawkins B (2008) Insect conservation: finding the way forward. Insect Conserv Divers 1(1):67–69. https://doi.org/10.1111/j.1752-4598.2007.00005.x
Lecq S, Loisel A, Bonnet X (2015) Non-lethal rapid biodiversity assessment. Ecol Ind 58:216–224. https://doi.org/10.1016/j.ecolind.2015.06.004
Lewis OT, New TR, Stewart AJA (2007) Insect conservation: progress and prospects. In: Stewart AJA, New TR, Lewis OT (eds) Insect conservation biology. CABI, Oxfordshire, pp 431–435
Lobo JM, Martín-Piera F, Veiga CM (1988) Las trampas pitfall con cebo, sus posibilidades en el estudio de las communidades coprofagas de Scarabaeidae (Col.). I. Caracteristicas determinantes de su capacidad de captura. Rev Ecol Biol Sol 25:77–100
Lobo JM (1993) Estimation of dung beetle biomass. Eur J Entomol 90:235–238
Lobo JM, Lumaret JP, Jay-Robert P (1998) Sampling dung beetles in the French Mediterranean area: effects of abiotic factors and farm practices. Pedobiologia 42:252–266
Lobo JM (2001) Decline of roller dung beetle (Scarabaeinae) populations in the Iberian peninsula during the 20th century. Biol Conserv 97(1):43–50. https://doi.org/10.1016/S0006-3207(00)00093-8
Lockwood J (1988) Not to harm a fly: our ethical obligations to Insects. Betw Species 4(3):204–211
Losey JE, Vaughan M (2006) The economic value of ecological services provided by insects. Bioscience 56(4):311–323. https://doi.org/10.1641/0006-3568(2006)56[311:TEVOES]2.0.CO;2
Lumaret JP, Lobo JM (1996) Geographic distribution of endemic dung beetles (Coleoptera, Scarabaeoidea) in the Western Palaearctic region. Biodivers Lett 3:192–199
Mächler E, Deiner K, Steinmann P, Altermatt F (2014) Utility of environmental DNA for monitoring rare and indicator macroinvertebrate species. Freshw Sci 33(4):1174–1183. https://doi.org/10.1086/678128
Madden AA, Barberán A, Bertone MA, Menninger HL, Dunn RR, Fierer N (2016) The diversity of arthropods in homes across the United States as determined by environmental DNA analyses. Mol Ecol 25(24):6214–6224. https://doi.org/10.1111/mec.13900

Mankin RW, Hagstrum DW, Smith MT, Roda AL, Kairo MTK (2011) Perspective and promise: a century of insect acoustic detection and monitoring. Am Entomol 57(1):30–44. https://doi.org/10.1093/ae/57.1.30

Manning P, Ford JP (2016) Evidence that sex-specific signals may support mate finding and limit aggregation in the dung beetle *Aphodius fossor*. Ecol Entomol 41(4):500–504. https://doi.org/10.1111/een.12319

Martín-Piera F, Lobo JM (1996) A comparative discussion of trophic preferences in dung beetle communities. Miscell Zool 19(1):13–31

McGeoch MA, Van Rensburg BJ, Botes A (2002) The verification and application of bioindicators: a case study of dung beetles in a savanna ecosystem. J Appl Ecol 39(4):661–672. https://doi.org/10.1046/j.1365-2664.2002.00743.x

Meyer A, Boyer F, Valentini A et al (2020) Morphological vs. DNA metabarcoding approaches for the evaluation of stream ecological status with benthic invertebrates: testing different combinations of markers and strategies of data filtering. Mol Ecol 00:1–18. https://doi.org/10.1111/mec.15723

New TR (1999) Untangling the web: spiders and the challenges of invertebrate conservation. J Insect Conserv 3(4):251–256. https://doi.org/10.1023/A:1009697104759

Nichols E, Spector S, Louzada J, Larsen T, Amezquita S, Favila ME, Network TSR (2008) Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. Biol Conserv 141(6):1461–1474. https://doi.org/10.1016/j.biocon.2008.04.011

Nichols E, Gardner TA (2011) Dung beetles as a candidate study taxon in applied biodiversity conservation research. In: Simmons LW, Ridsdill-Smith J (eds) Ecology and evolution of dung beetles. Princeton University Press, Princeton, pp 267–291

Noriega JA, March-Salas M, Pertierra LR, Vulinec K (2020) Spatial partitioning of perching on plants by tropical dung beetles depends on body size and leaf characteristics: a sit-and-wait strategy for food location. Ecol Entomol 45(5):1108–1120. https://doi.org/10.1111/een.12898

Oksanen J, Blanchet FG, Kindt R et al (2015) Vegan: community ecology package. R package version 2.2–1

Paulian R, Baraud J (1982) Faune des Coléoptères de France, vol. II : Lucanoidea et Scarabaeoidea. Paris, Lechevalier

Pimm SL, Alibhai S, Bergl R et al (2015) Emerging technologies to conserve biodiversity. Trends Ecol Evol 30(11):685–696. https://doi.org/10.1016/j.tree.2015.08.008

Port JA, O'Donnell JL, Romero-Maraccini OC, Leary PR, Litvin SY, Nickols KJ et al (2016) Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA. Mol Ecol 25(2):527–541. https://doi.org/10.1111/mec.13481

Pont D, Rocle M, Valentini A, Civade R, Jean P, Maire A, Dejean T (2018) Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. Sci Rep 8(1):1–13. https://doi.org/10.1038/s41598-018-28424-8

Potamitis I, Schäfer P (2014) On classifying insects from their wingbeat: New results. Ecology and acoustics: emergent properties from community to landscape, meeting papers, 16–18.

Purvis A, Hector A (2000) Getting the measure of biodiversity. Nature 405:212–219. https://doi.org/10.1038/35012221

R Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rees HC, Maddison BC, Middleditch DJ, Patmore JRM, Gough KC (2014) The detection of aquatic animal species using environmental DNA: a review of eDNA as a survey tool in ecology. J Appl Ecol 51(5):1450–1459. https://doi.org/10.1111/1365-2664.12306

Régnier C, Achaz G, Lambert A, Cowie RH, Bouchet P, Fontaine B (2015) Mass extinction in poorly known taxa. Proc Natl Acad Sci USA 112(25):7761–7766. https://doi.org/10.1073/pnas.1502350112

Rössner E, Schönfeld J, Ahrens D (2010) *Onthophagus (Palaeonthophagus) medius* (Kugelann, 1792)—a good western palaearctic species in the *Onthophagus vacca* complex (Coleoptera: *Scarabaeidae*: *Scarabaeinae*: *Onthophagini*). Zootaxa 2629:1–28

Samways MJ, Barton PS, Birkhofer K, Chichorro F, Deacon C, Fartmann T, Fukushima CS, Gaigher R, Habel JC, Hallmann CA, Hill MJ, Hochkirch A, Kaila L, Kwak ML, Maes D, Mammola S, Noriega JA, Orfinger AB, Pedraza F, Pryke JS, Roque FO, Settele J, Simaika JP, Stork NE, Suhling F, Vorster C, Cardoso P (2020) Solutions for humanity on how to conserve insects. Biol Conserv 242:108427. https://doi.org/10.1016/j.biocon.2020.108427

Sheppard SK, Harwood JD (2005) Advances in molecular ecology: tracking trophic links through predator-prey food-webs. Funct Ecol 19(5):751–762. https://doi.org/10.1111/j.1365-2435.2005.01041.x

Simmons LW, Ridsdill-Smith J (2011) Ecology and evolution of dung beetles. Princeton University Press, Princeton

Small E (2012) The new Noah's Ark: beautiful and useful species only. Part 2. The chosen species. Biodiversity 13(1):37–53. https://doi.org/10.1080/14888386.2012.659443

Spector S (2006) Scarabaeine dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae): an invertebrate focal taxon for biodiversity research and conservation. Coleopt Bull 60(mo5):71–83. https://doi.org/10.1649/0010-065X(2006)60[71:SDBCSS]2.0.CO;2

Taberlet P, Coissac E, Hajibabaei M, Rieseberg LH (2012) Environmental DNA. Mol Ecol 21(8):1789–1793. https://doi.org/10.1111/j.1365-294X.2012.05542.x

Takahara T, Minamoto T, Yamanaka H, Doi H, Kawabata ZI (2012) Estimation of fish biomass using environmental DNA. PLoS ONE 7(4):e35868. https://doi.org/10.1371/journal.pone.0035868

Taylor HR, Gemmell NJ (2016) Emerging technologies to conserve biodiversity: further opportunities via genomics. Response to Pimm et al. Trends Ecol Evol 31(3):171–172. https://doi.org/10.1016/j.tree.2016.01.002

Taylor HR, Dussex H, van Heezik Y (2017) Bridging the conservation genetics gap by identifying barriers to implementation for conservation practitioners. Glob Ecol Conserv 10:231–242. https://doi.org/10.1016/j.gecco.2017.04.001

Thomsen PF, Kielgast J, Iversen LL et al (2012) Monitoring endangered freshwater biodiversity using environmental DNA. Mol Ecol 21(11):2565–2573. https://doi.org/10.1111/j.1365-294X.2011.05418.x

Thomsen PF, Willerslev E (2015) Environmental DNA: an emerging tool in conservation for monitoring past and present biodiversity. Biol Conserv 183:4–18. https://doi.org/10.1016/j.biocon.2014.11.019

Thomsen PF, Sigsgaard EE (2019) Environmental DNA metabarcoding of wild flowers reveals diverse communities of terrestrial arthropods. Ecol Evol 9(4):1665–1679. https://doi.org/10.1002/ece3.4809

Tocco C, Villet M (2016) Dung beetle (Coleoptera: Scarabaeoidea) assemblages in the western Italian Alps: benchmark data for land use monitoring. Biodivers Data Journal 4:e10059. https://doi.org/10.3897/BDJ.4.e10059

Valentini A, Taberlet P, Miaud C, Civade R, Herder J, Thomsen PF et al (2016) Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. Mol Ecol 25(4):929–942. https://doi.org/10.1111/mec.13428

Veiga CM, Lobo JM, Martín-Piera F (1989) Las trampas pitfall con cebo, sus posibilidades en el estudio de las comunidades coprofagas de Scarabaeoidea (Col.) II. Analisis de efectividad. Rev Écol Biol Sol 26(1):91–109

Verdú JR, Lobo JM, Sánchez-Piñero F, Gallego B, Numa C, Lumaret JP et al (2018) Ivermectin residues disrupt dung beetle diversity, soil properties and ecosystem functioning: an interdisciplinary

field study. Sci Total Environ 618:219–228. https://doi.org/10.1016/j.scitotenv.2017.10.331

Viegas G, Stenert C, Schul UH, Maltchik L (2014) Dung beetle communities as biological indicators of riparian forest widths in southern Brazil. Ecol Ind 36:703–710. https://doi.org/10.1016/j.ecolind.2013.09.036

Watts C, Dopheide A, Holdaway R, Davis C, Wood J, Thornburrow D, Dickie IA (2019) DNA metabarcoding as a tool for invertebrate community monitoring: a case study comparison with conventional techniques. Austral Entomol 58(3):675–686. https://doi.org/10.1111/aen.12384

Wurmitzer C, Blüthgen N, Krell FT, Maldonado B, Ocampo F, Müller JK, Schmitt T (2017) Attraction of dung beetles to herbivore dung and synthetic compounds in a comparative field study. Chemoecology 27(2):75–84. https://doi.org/10.1007/s00049-017-0232-6

Xu CCY, Yen IJ, Bowman D, Turner CR (2015) Spider Web DNA: a new spin on noninvasive genetics of predator and prey. PLoS ONE 10(11):e0142503. https://doi.org/10.1371/journal.pone.0142503