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# Biodiversity assessments in the 21st century: The potential of insect traps to complement environmental samples for estimating eukaryotic and prokaryotic diversity using high-throughput DNA metabarcoding



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| Complete List of Authors: | Ritter, Camila; University of Duisburg-Essen, Department of Eukaryotic Microbiology<br>Häggqvist , Sibylle ; Swedish Museum of Natural History, Department of Zoology, Box 50007, 104 05 Stockholm, Sweden<br>Sääksjärvi , Ilari; Biodiversity Unit, University of Turku, FI-20014 Turku, Finland<br>Karlsson, Dave; Station Linné, Skogsby 161, 386 94 Färjestaden, Sweden<br>Muasya, Muthama; University of Cape Town Department of Biological Sciences<br>Nilsson, R. Henrik; Goteborgs Universitet, Biological and Environmental Sciences<br>Antonelli, Alexandre; Goteborgs Universitet, Biological and Environmental Sciences |





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# Biodiversity assessments in the 21st century: The potential of insect traps to complement environmental samples for estimating eukaryotic and prokaryotic diversity using high-throughput DNA metabarcoding

Camila D. Ritter[1*], S. Häggqvist[2], D. Karlsson[3], I.E. Sääksjärvi[4], A. Muthama Muasya[5], R. Henrik Nilsson[6,7], Alexandre Antonelli[6, 7, 8,]

[1]Department of Eukaryotic Microbiology, University of Duisburg-Essen, Universitätsstrasse 5 S05 R04 H83 D-45141 Essen, Germany

[2]Swedish Museum of Natural History, Department of Zoology, Box 50007, 104 05 Stockholm, Sweden

[3]Station Linné, Skogsby 161, 386 94 Färjestaden, Sweden

[4]Biodiversity Unit, University of Turku, FI-20014 Turku, Finland

[5]Department of Biological Sciences, University of Cape Town, Rondebosch 7701, RSA

[6]Gothenburg Global Biodiversity Centre, Box 461, SE-405 30 Göteborg, Sweden

[7]Department of Biological and Environmental Sciences, University of Gothenburg, Box 463, 405 30 Göteborg, Sweden

[8]Gothenburg Botanical Garden, Carl Skottsbergs gata 22A, SE-41319 Göteborg, Sweden

*Corresponding author: Camila D. Ritter kmicaduarte@gmail.com.
kmicaduarte@gmail.com, camila.ritter@gu.se Phone: +55 48991434597.

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## Abstract

The rapid loss of biodiversity, coupled with difficulties in species identification, call for innovative approaches to assess biodiversity. Insects make up a substantial proportion of extant diversity and play fundamental roles in any given ecosystem. To complement morphological species identification, new techniques such as metabarcoding make it possible to quantify insect diversity and insect-ecosystem interactions through DNA sequencing. Here we examine the potential of bulk insect samples (i.e., containing many non-sorted specimens) to assess prokaryote and eukaryote biodiversity and to complement the taxonomic coverage of soil samples. We sampled 25 sites on three continents and in various ecosystems, collecting insects with Slam-traps (Brazil) and Malaise-traps (South Africa and Sweden). We then compared our diversity estimates with the results obtained with biodiversity data from soil samples from the same localities. We found a largely different taxonomic composition between the soil and insect samples, testifying to the potential of bulk insect samples to complement soil samples. Finally, we found that non-destructive DNA extraction protocols, which preserve insect specimens for morphological studies, constitute a promising choice for cost-effective biodiversity assessments. We propose that the sampling and sequencing of insect samples should become a standard complement for biodiversity studies based on environmental DNA.


**Key-words:** Environmental DNA, COI mtDNA, Non-destructive DNA extraction, Metabarcoding, 16S rDNA, 18S rDNA.

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**Background**

Quantification of biological diversity is crucial for many biological and societal applications, including biogeography and ecosystem services, understanding biological interactions, and for designing conservation and management strategies. But quantifying biodiversity is a big challenge in that it requires taxonomic expertise, substantial time, and significant funding (Campbell et al. 2011). Recent molecular (DNA-based) tools, however, hold the potential to speed up biodiversity quantification manifold while keeping costs down. With methods such as DNA metabarcoding (Taberlet et al. 2012a), it is now possible to quantify the genetic diversity of any locality without the need to spend years examining specimens and their morphological characters (Gibson et al. 2014; Bush et al. 2017; Lugg et al. 2017).

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Metabarcoding techniques have been used successfully for species identification from bulk organism samples (e. g. Taberlet et al. 2012b; Liu et al. 2013) as well as from environmental samples of soil, litter, faeces, and water (Taberlet et al. 2012a; Lanzen et al. 2016). Insects represent more than 50% of the described eukaryotic diversity (Stork et al. 2015; Stork 2018) and are essential for ecosystem functioning (e. g. Bascompte et al. 2003; Calvignac-Spencer et al. 2013; Miller et al. 2016). Applying metabarcoding methods to insect samples may allow us not only to assess insect biodiversity *per se,* but also the prokaryote and eukaryotic diversity in those samples, including organisms present on and inside the insect bodies and any food remains in their digestive system. For instance, Schnell et al. (2015) used hematophagous leeches to estimate the diversity of vertebrates in the region where the leeches were sampled.

To test the usefulness of bulk insect samples as environmental DNA (eDNA) in different environments, we sampled insects and soil on three continents (Europe, Africa, and South America). We sequenced two fragments of the ribosomal genes using general primers for prokaryotes (the 16S gene) and eukaryotes (the 18S gene). We tested six different DNA extraction protocols, of which three were destructive and three were non-destructive (preserving the exoskeleton of insects), to assess the efficiency of non-destructive protocols to register other organisms beyond insects. The goals of this study are to i) evaluate the taxonomic coverage that can be attained through metabarcoding of insect samples; ii) test the feasibility of a range of lab protocols and sampling techniques on samples from various environments, ranging from tropical rainforests to temperate grasslands; and iii) based on the results from (i) and (ii), propose a standardized protocol for collecting and processing soil and insect samples, including the prokaryotic and eukaryotic organisms associated with the insects. Our unified framework facilitates the assessment of a large portion of the total biodiversity of any site and thereby complements traditional taxonomic inventories.

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## Material and Methods

*Sampling design:* Soil samples were collected in three countries: Brazil, South Africa, and Sweden following the protocol described in Tedersoo et al. (2014) and Ritter et al. (2018). Arthropods, mainly flying insects, were collected in the same localities with Slam-traps (Brazil) or Malaise-traps (South Africa and Sweden). Both are tent-like traps made of fine mesh-netting, widely used in entomological studies and aimed at capturing strong-flying insects (e.g. wasps, mosquitos and butterflies) that typically fly upwards after hitting a fine-scale net, and which are ultimately trapped in a bottle filled with ethanol or another preserving liquid. These two traps differ mainly in shape, with Slam-traps resembling an igloo (dome-

shaped) and Malaise-traps a Canadian tent (higher on one end). Figure 1 summarizes our sampling design and shows examples of these two insect traps. The sampling locations are shown in Fig. 2. Sampling time differed among countries due to logistic conditions: in Brazil and South Africa we were just able to keep the traps open for one day, whereas in Sweden we were able to keep them open for seven days. The details of the sampling localities are summarized in Table 1.

*DNA extraction:* We first tested the efficiency of five DNA extraction protocols on five insect samples obtained from Sweden (Table S1). Since we did not find significant differences between the protocols in either an ANOVA test (with the total number of OTUs; $p = 0.96$) or a Kruskal-Wallis test (with the number of OTUs by taxonomic group; $p = 0.66$), we decided to perform all subsequent analyses using the non-destructive protocol of Aljanabi and Martinez (1997) for insect sample DNA extractions. The samples were immersed in 15 ml of salt buffer (0. 4 M NaCl, 10 mM Tris-HCl pH = 8, and 2mM EDTA pH = 8) using a vortex mixer for 1 min. Then 1.5 ml of 20% SDS and 20 µl of 20mg/ml Proteinase K were added. Whenever the insects were not completely covered by the buffer, we added additional buffer of the same proportion of reagents until all insects were covered. The samples were incubated at 60°C overnight. After that, we transferred 15 ml of clear lysis solution into a new tube and the insects were transferred into 99% ethanol for preservation. Then, 11.25 ml of 6 M NaCl were added to the lysis solution, and the samples were vortexed for 30 s at maximum speed. The samples were then centrifuged at 10,000 g for 30 min, and 25 ml of the supernatant were transferred to a new tube. An equal volume of isopropanol was added. The samples were incubated at -20°C for 1 h, and then centrifuged at 10,000 g for 20 min at 4°C. Pellets were washed with 2 ml of 70% ethanol, dried in room temperature, and re-suspended in 300 µl of sterile $dH_2O$. For soil samples, 10 grams of dried soil were used for extraction with the

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PowerMax® Soil DNA Isolation Kit (MO BIO Laboratories, USA), following the manufacturer's instructions as detailed in Ritter et al. (2018).

*PCR amplification:* The amplification and sequencing of the nuclear 16S, 18S and cytochrome c oxidase subunit I mitochondrial (COI) genes regions were performed by Macrogen (Republic of Korea). For 16S we targeted the V3-V4 region (~460) using the forward primer (5'- CCTACGGGNGGCWGCAG - 3') and reverse primer (5'-GACTACHVGGGTATCTAATCC - 3') from Klindworth et al. (2013), following standard protocols of Macrogen (South Korea). Sequencing was performed using the Illumina MiSeq 2x300 platform. For metabarcoding of the 18S region, we targeted the hypervariable V7 region of the 18S rRNA gene using the 5'-TTTGTCTGSTTAATTSCG-3' and 5'-TCACAGACCTGTTATTGC-3' forward and reverse primers designed by Guardiola et al. (2015) and later tested soil samples in French Guiana (Zinger et al. 2017), which yield a 100–110 long fragment. Sequencing was done using the Illumina MiSeq 2x250 platform. For COI, we amplified a region of ~313 using an internal forward primer (5' - GGWACWGGWTGAACWGTWTAYCCYCC - 3', Leray et al. 2013) and the COI degenerate reverse primer (5' – TAAACTTCAGGGTGACCAAARAAYCA - 3', Folmer et al. 1994). Sequencing was carried out using the Illumina MiSeq 2x300 platform.

*Operational taxonomic unit (OTU) assessment:* OTU selection for each library was performed using the USEARCH/UPARSE v9. 0. 2132 (Edgar 2013) Illumina paired reads pipeline. We filtered the sequences by quality to discard chimeras and clustered sequences into OTUs at a minimum similarity of 97% using a "greedy" algorithm that performs chimera filtering and OTU clustering simultaneously (Edgar 2013). Singletons were removed. We

used SILVAngs 1. 3 for taxonomic assignments of the OTUs of 16S and 18S, using a representative sequence from each OTU for all insects and soil samples. The reference data were SINA v1.2.10 for ARB SVN (revision 21008) and BLAST (Altschul et al. 1997) for the classification of sequences (Camacho et al. 2009). For COI, we used the sequences available in GenBank (Benson et al. 2005) and blasted them with the "blastn" tool.

*Statistical analysis:* We first rarefied all samples to equal depth, where the depth was determined by the lowest number of reads obtained from a single plot (Fig. 3). We also estimated the Chao1 richness for each plot based on the original data with the number of reads, using the "estimateR" function (Table 2). As the Chao1 estimates and the rarefied richness were high correlated (the lowest correlation was observed for insect samples of the COI gene [0.83], whereas the highest correlation was observed for soil samples of the COI gene [0.99]) we just used the rarefied data in the subsequent analyses (Table S2). We subsequently transformed the OTU tables to presence/absence for both prokaryotic (16S) and eukaryotic (18S) data. We employed two methods to test the difference among samples. First, we used a permutational multivariate analysis of variance (PERMANOVA) test with country, habitat, and kind of sample (insects or soil) as predictors, and dissimilarity matrices using the Jaccard index of OTUs from prokaryotes (16S), eukaryotes (18S) and eukaryotes (COI) as response variables. Second, we ran a two-dimensional Non-metric Multidimensional Scaling (NMDS) ordination of all samples (Legendre and Legendre 1998) with the Jaccard distance matrix. In this run we used the "envfit" method to fit country, habitat type, and kind of sample onto the NMDS ordination as a measure of the correlation of these factors with the NMDS axes. The function "envfit" finds vectors or factor averages of environmental variables. Vector fitting is a regression and as a regression the $R^2$ is easily computed. All analyses were

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made in the VEGAN package version v. 2. 4-3 (Oksanen et al. 2007) run in the R environment R v3.4.2 (R Development Core Team 2017).

## Results

After rarefaction, the prokaryotic (16S) data presented a total of 10,679 OTUs from the soil samples and 5,721 OTUs from the insect samples. Of these, we were able to assign 10,492 (98%, soils) and 5,334 (93%, insects) to a taxonomic affiliation at or below the order level. For eukaryotes (18S) we obtained 9,508 OTUs (soil samples) and 2,168 OTUs (insect samples), of which 8,344 (88%) and 1,929 (89%) were assignable to the order level or below, respectively. For eukaryotes (COI) we obtained 6,611 OTUs (soil samples) and 3,287 OTUs (insect samples), of which 3,748 (57%) and 1,812 (56%) were assignable to the order level or below, respectively. Tables with the number of reads of each OTU by plot, and their taxonomic assignments, are provided in the supplementary material (S3 – S5).

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The taxonomic composition of prokaryotes and eukaryotes was different between the soil and the insect samples (Fig. 4). For the prokaryotic component of the soil samples, the highest number of OTUs was found to stem from Proteobacteria (~25%, mostly Alphaproteobacteria), Actinobacteria (~15%), and Acidobacteria (~10%, Fig. 3). For the insect samples, however, the corresponding order was Proteobacteria (~30%), Bacteroides (~20%), and Actinobacteria (~12%, Fig. 3). For eukaryotes (18S) from the soil samples, the group with the highest number of OTUs was Fungi (~25%, mainly Ascomycota and Basidiomycota) followed by Rizharia (~15%) and Alveolata and Amebozoa (both with ~10%, Fig. 4). For the insect samples, and apart from the obvious dominance of insects due to the

highest insect biomass in the samples (~70%), we found a relatively high proportion of Fungi (~15%), Arachnida, Chloroplastida (Viridiplantae), and Alveolata (all with ~5%, Fig. 4). Interestingly, for COI we found similar proportions of taxonomic groups in soil and insect samples (Fig. 4), with the highest number of "unknown" OTUs (~40%). The assigned group with the highest number of OTUs was Fungi (~30%, mainly Ascomycota and Basidiomycota), followed by Hexapoda and Stramenopiles (both with ~10% in soil samples and Stramenopiles with ~5% in insect samples, Fig. 4).

No clear difference in taxonomic composition considering orders was observed with respect to habitat types for either the prokaryote or the eukaryote datasets. With respect to the number of OTUs belonging to insects, the decreasing order of OTUs in the insect samples was Sweden (794 [18S] and 176 [COI]), Brazil (561 [18S] and 82 [COI]) and South Africa (405 [18S] and 44 [COI]). The most common insect orders in soil and insect samples were Coleoptera, Diptera, Hymenoptera, and Lepidoptera. The number of OTUs for each order per marker and sample type are summarized in Table 3.

The PERMANOVA results showed a significant effect for country (16S [$R^2 = 0.16$, $p < 0.001$], 18S [$R^2 = 0.14$, $p < 0.001$], COI [$R^2 = 0.13$, $p < 0.001$]) and type of sample (16S [$R^2 = 0.22$, $p < 0. 001$], 18S [$R^2 = 0.22$, $p < 0.001$], COI [$R^2 = 0.08$, $p < 0.001$]) for all the prokaryote (16S), the eukaryotic (18S) and (COI) communities. The results of the NMDS showed a clear separation in community composition among the plots by country and type of sample (Fig. 5). The envfit test indicated significant effects of country on the communities of prokaryotes ($R^2 = 0. 16$, $p = 0.007$) and eukaryotes based on COI ($R^2 = 0. 42$, $p = 0.001$), but not for eukaryotes based on 18S ($R^2 = 0. 10$, $p = 0.055$). However, for the latter relationship we note that the p-value of 0.055 is close to the arbitrary alpha value of significance, which

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indicates at least a tendency of difference among countries. The envfit test also indicated a significant effect for kind of sample on the prokaryote ($R^2$ = 0. 63, $p < 0.001$), eukaryote 18S ($R^2$ = 0.79, $p < 0.001$) and eukaryote COI ($R^2$ = 0.40, $p < 0.001$) communities. For habitat type, the effect was just significant for eukaryotic COI communities ($R^2$ = 0.43, $p < 0.001$).

## Discussion

In this study we show that sequencing both bulk insect samples and soils in a unified framework (including the gene regions 16S, 18S, and COI) allows the assessment of a large portion of the prokaryotic and eukaryotic diversity of any location. A previous comparison between soil and insect samples was done by Yang et al. (2014), however with an exclusive focus on insects collected in these different samples. Interestingly, between 22% and 40% of the OTUs recovered in our eukaryotic 18S data were found to stem from other groups than insects (such as Arachnida, Fungi, and various organisms of the protist type), indicating that our approach allows the identification of organisms associated with insects and provides a cost-effective identification tool for multiple taxonomic groups. This result was even stronger in the COI data, where around 83% of the assigned OTUs belonged to other groups than insects.

Significant differences in community composition were detected between soil and insect samples for both the prokaryote and the eukaryote datasets. These results support the use of bulk insect samples to complement environmental biodiversity assessments based on soils. Insect trapping is known to primarily recover living organisms, whereas soil samples often retrieve a significant proportion of dead organisms and legacy DNA (Carini et al. 2016). For the insect trapping dataset, we know that we targeted living organisms present in the

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environment at the time of sampling, since adult insects have a short life span, usually around a few weeks or even days. This offers the possibility of drawing a direct connection between diversity estimates, time of the year, and even climatic conditions under which those insects are active. It also minimizes the risk of sequencing organisms no longer living in the locality but still detectable in the form of dead tissue, which happens in soil samples (Baldrian et al. 2012; Creer et al. 2016).

The non-significant difference among habitat types for both the prokaryote (16S) and eukaryote (18S) datasets is probably due to the low number of replicates and the high number of habitat types surveyed. However, the COI data showed a significant difference among habitat types. This result highlights the usefulness of insect samples in any environment, since our results portrayed different taxonomic profiles in the insect samples and the corresponding soil samples across all habitat types surveyed. For insect samples, Sweden registered the highest number of OTUs for insects (794 [18S] and 176 [COI]) compared to Brazil (561 [18S] and 82 [COI]) and South Africa (405 [18S] and 44 [COI]). This ranking of absolute numbers is most probably related to the sampling design, since in Sweden the traps were open for seven days, compared to a single day in Brazil and South Africa for logistic reasons. In mega-diverse habitats such as Amazonia, a comprehensive long-term insect diversity sampling may take months or even years and should be carried out with multiple collecting methods (Gómez et al. 2018; Matos-Maraví et al. 2018). However, in light of logistic and financial limitations, fast massive sampling such as one week or even one day may allow a rather substantial biodiversity registration, especially when compared to a one-day traditional inventory of macro-organisms such as vertebrates or plants.

Universal primers, such as the 18S primers we used, are capable of detecting the majority of eukaryote organisms. However, the 18S is not variable enough to distinguish all eukaryotes at

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the species level (Hartmann et al. 2010; Lindahl et al. 2013). The use of other gene regions or markers might extend the taxonomic groups detected. Suitable additional markers include the nuclear internal transcribed spacer region (ITS) for fungi (Tedersoo et al. 2014) and plants (Chen et al. 2010). However, the incomplete nature of sequence databases, combined with the short length of the regions sequenced, also pose a problem for our COI data, despite its widespread use in initiatives such as the International Barcode of Life project (http://ibol.org/). Although we used general primers for metazoans (Hebert et al. 2003), we still found that around 40% of the OTUs could not be assigned even at the phylum level. On the other hand, most insects in the COI dataset were identified at least to the family level, while with the 18S dataset most matchings could be made at the order level (tables S3 – S5). Using markers with better taxonomic resolution or more specific for target groups, the bulk insect samples could also be used for monitoring rare or hard-to-sample groups, for instance mammals (Kocher et al. 2017), or network interactions such as plant-insects (Kergoat et al. 2017) and host-parasite interactions (Toju 2015).

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Based on our tests, we recommend the use of non-destructive protocols, which allow subsequent use of the insects and other organisms captured in the ethanol samples for morphological studies, vouchering in museums, photographic documentation, and re-sequencing for other genetic markers. Given that the vast majority of extant insects remains to be described (Stork 2018), it is promising that non-destructive DNA extraction protocols allow molecular studies from bulk insect samples. Any interesting insect lineages recovered in the DNA sequencing stage can then be tracked back to the bulk sample where it comes from, located manually, and then studied further using traditional morphological means, with only small losses (e. g., some color fading and increased fragility of specimens). This possibility also protects the species discovery potential of metabarcoding efforts (e. g. Nilsson et al. 2016).

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**Unified protocol.** Based on the results obtained and our experience in field over many years and environments, we suggest a general sampling protocol for any student, researcher, or practitioner aiming at assessing diversity across locations (Appendix 1, Supplementary material). We recommend the sampling of insects using Malaise traps for at least a week for rapid biodiversity assessment. Studies seeking to do a taxonomic survey of one particular locality should take seasonal variations into account, ideally by sampling a few times per year. We also recommend the use of non-destructive DNA extraction protocols to allow complementary taxonomic studies and documentation, such as the one used here. Traditional taxonomic assessments remain crucial for the identification and description of new species, but it is clear from the present study that both techniques – molecular and morphological – are complementary and should be combined in future efforts, whenever possible.

## Conclusions

In this study we show the value of collecting and sequencing bulk insect samples to complement soil samples in rapid biodiversity assessments, thereby capturing a much larger proportion of a site's prokaryote and eukaryote diversity than traditional methods during the same time allocation. Another important advantage of the use of bulk insect samples is that it can be used in a wide range of environments, from hot and humid rainforests, to species-rich Mediterranean meadows, and natural and anthropogenic habitats in the temporal zone. The use of non-destructive DNA extraction also allows complementary taxonomic studies, speeding up the discovery of new species (e. g. Gómez et al. 2018). Adopting a massive and standardised sampling scheme would allow fast and cost-effective estimations of global biodiversity.

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**Data Avalailiability and Accessibility -** Raw data with the DNA sequences are under Bioproject 410648; BioSample accession SAMN09081757 - SAMN09081906; NCBI SRA: PRJNA464362. OTUs tables and R-script used in the analysis are in the Supplementary Material.

**Conflicts of interest** – The authors declare that they have no conflicts of interest.

**Permit(s)** – Permits for this research were provided by the Brazilian authorities ICMBio (register number 48185-2) and IBAMA (register number 127341), and by the South Africa authority CapeNature (register number 0056-AAA041-00150).

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1 **Tables:**

2 **Table 1: Description of the sampling localities.** Country, type of environment (Vegetation), longitude, latitude, date of sampling (Date), kind of
3 trap used to collect insects, period of time the traps were opened (Time of sampling), highest and coldest average temperature, and precipitation
4 average of the sampled month (extracted from https://pt.weatherspark.com/) for each plot.

5

| Country | Locality | Vegetation | Long | Lat | Date | Trap | Time of sampling | Average temp. | | Average prec. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Highest | Coldest | |
| Brazil | BC IG P1 | Seasonally flooded tropical forest | -70.01 | -4.33 | Nov-15 | Slam-trap | 24 h. | 29°C | 22°C | 129 mm/month |
| Brazil | BCIGP2 | Seasonally flooded tropical forest | -70.00 | -4.32 | Nov-15 | Slam-trap | 24 h. | 29°C | 22°C | 129 mm/month |
| Brazil | BCIGP3 | Seasonally flooded tropical forest | -70.00 | -4.32 | Nov-15 | Slam-trap | 24 h. | 29°C | 22°C | 129 mm/month |
| Brazil | BCTFP1 | Unflooded tropical forest | -70.07 | -4.43 | Nov-15 | Slam-trap | 24 h. | 29°C | 22°C | 129 mm/month |

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|---|---|---|---|---|---|---|---|---|---|---|
| Brazil | BCTFP2 | Unflooded tropical forest | -70.06 | -4.43 | Nov-15 | Slam-trap | 24 h. | 29°C | 22°C | 129 mm/month |
| Brazil | BCTFP3 | Unflooded tropical forest | -70.08 | -4.43 | Nov-15 | Slam-trap | 24 h. | 29°C | 22°C | 129 mm/month |
| Brazil | BCVZP1 | Seasonally flooded tropical forest | -70.01 | -4.34 | Nov-15 | Slam-trap | 24 h. | 29°C | 22°C | 129 mm/month |
| Brazil | BCVZP2 | Seasonally flooded tropical forest | -70.02 | -4.35 | Nov-15 | Slam-trap | 24 h. | 29°C | 22°C | 129 mm/month |
| Brazil | BCVZP3 | Seasonally flooded tropical forest | -70.03 | -4.35 | Nov-15 | Slam-trap | 24 h. | 29°C | 22°C | 129 mm/month |
| South Africa | SAPC1P1 | Arid-mountain area | 18.48 | -33.51 | Aug-16. | Malaise-trap | 24 h. | 18°C | 10°C | 24 mm/month |
| South Africa | SAPC2P1 | Dry coastal-area | 18.23 | -31.80 | Aug-16. | Malaise-trap | 24 h. | 18°C | 10°C | 24 mm/month |
| South Africa | SAPC2P2 | Dry coastal-area | 18.24 | -31.81 | Aug-16. | Malaise-trap | 24 h. | 18°C | 10°C | 24 mm/month |
| South Africa | SAPC2P3 | Dry coastal-area | 18.23 | -31.79 | Aug-16. | Malaise-trap | 24 h. | 18°C | 10°C | 24 mm/month |

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|---|---|---|---|---|---|---|---|---|---|---|
| South Africa | SAPC3P1 | Dry coastal-area | 19.20 | -34.43 | Aug-16. | Malaise-trap | 24 h. | 18°C | 10°C | 24 mm/month |
| South Africa | SAPC3P2 | Dry coastal-area | 19.20 | -34.43 | Aug-16. | Malaise-trap | 24 h. | 18°C | 10°C | 24 mm/month |
| South Africa | SAPC3P3 | Dry coastal-area | 19.21 | -34.43 | Aug-16. | Malaise-trap | 24 h. | 18°C | 10°C | 24 mm/month |
| Sweden | SEFORP1 | Temperate forest fragment | 13.66 | 58.44 | Jun-16 | Malaise-trap | Seven days | 21°C | 10°C | 52 mm/month |
| Sweden | SEFORP2 | Temperate forest fragment | 13.64 | 58.40 | Jun-16 | Malaise-trap | Seven days | 21°C | 10°C | 52 mm/month |
| Sweden | SEFORP3 | Temperate forest fragment | 13.74 | 58.49 | Jun-16 | Malaise-trap | Seven days | 21°C | 10°C | 52 mm/month |
| Sweden | SEARAP1 | Arable area | 13.76 | 58.52 | Jun-16 | Malaise-trap | Seven days | 21°C | 10°C | 52 mm/month |
| Sweden | SEARAP2 | Arable area | 13.66 | 58.44 | Jun-16 | Malaise-trap | Seven days | 21°C | 10°C | 52 mm/month |
| Sweden | SEARAP3 | Arable area | 13.62 | 58.38 | Jun-16 | Malaise-trap | Seven days | 21°C | 10°C | 52 mm/month |
| Sweden | SEPASP1 | Pasture area | 13.74 | 58.40 | Jun-16 | Malaise-trap | Seven days | 21°C | 10°C | 52 mm/month |

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|---|---|---|---|---|---|---|---|---|---|---|
| Sweden | SEPASP2 | Pasture area | 13.66 | 58.44 | Jun-16 | Malaise-trap | Seven days | 21°C | 10°C | 52 mm/month |
| Sweden | SEPASP3 | Pasture area | 13.74 | 58.49 | Jun-16 | Malaise-trap | Seven days | 21°C | 10°C | 52 mm/month |

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**Table 2: Chao 1 estimates per plot.** Observed richness (OR), Chao1 estimates, and standard error (SE) per plot for each marker: prokaryotes (16S) and eukaryotes (18S and COI), and for each kind of sample (Bulk insect samples and soil samples).

| | 16S | | | | | | 18S | | | | | | COI | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Soil | | | Insects | | | Soil | | | Insects | | | Soil | | | Insects | | |
| **Plot** | **OR** | **Chao1** | **SE** | **OR** | **Chao1** | **SE** | **OR** | **Chao1** | **SE** | **OR** | **Chao1** | **SE** | **OR** | **Chao1** | **SE** | **OR** | **Chao1** | **SE** |
| SBCIGP1 | 1488 | 1768 | 41 | 558 | 632 | 22 | 1156 | 1328 | 32 | 200 | 238 | 15 | 164 | 243 | 34 | 137 | 143 | 5 |
| SBCIGP2 | 1594 | 1973 | 51 | 420 | 469 | 19 | 1066 | 1204 | 26 | 172 | 213 | 17 | 180 | 239 | 29 | 161 | 169 | 6 |
| SBCIGP3 | 1576 | 1926 | 45 | 655 | 720 | 21 | 1048 | 1142 | 21 | 174 | 221 | 18 | 235 | 290 | 27 | 125 | 129 | 4 |
| SBCTFP1 | 1472 | 1740 | 37 | 1357 | 1633 | 38 | 1041 | 1191 | 28 | 129 | 156 | 13 | 253 | 290 | 19 | 109 | 119 | 10 |
| SBCTFP2 | 1457 | 1684 | 33 | 585 | 636 | 19 | 1087 | 1231 | 27 | 270 | 305 | 13 | 194 | 225 | 17 | 116 | 119 | 4 |
| SBCTFP3 | 1187 | 1445 | 39 | 509 | 548 | 17 | 1193 | 1281 | 18 | 232 | 281 | 16 | 209 | 240 | 15 | 171 | 184 | 10 |
| SBCVZP1 | 1428 | 1739 | 43 | 422 | 476 | 24 | 857 | 982 | 26 | 207 | 242 | 14 | 145 | 183 | 19 | 134 | 142 | 8 |
| SBCVZP2 | 1598 | 1945 | 46 | 939 | 1038 | 27 | 1083 | 1236 | 29 | 274 | 304 | 13 | 154 | 184 | 16 | 114 | 122 | 5 |
| SBCVZP3 | 1441 | 1763 | 44 | 413 | 444 | 14 | 1056 | 1190 | 26 | 245 | 322 | 24 | 146 | 188 | 21 | 101 | 119 | 16 |
| SSAPC1P1 | 2181 | 2820 | 67 | 413 | 431 | 9 | 1170 | 1233 | 16 | 209 | 252 | 17 | 431 | 436 | 4 | 155 | 164 | 9 |
| SSAPC2P1 | 2401 | 2690 | 34 | 692 | 739 | 18 | 945 | 1042 | 26 | 196 | 207 | 6 | 475 | 496 | 12 | 64 | 97 | 26 |
| SSAPC2P2 | 2038 | 2399 | 43 | 323 | 352 | 17 | 1085 | 1154 | 18 | 64 | 75 | 9 | 631 | 644 | 8 | 48 | 48 | 0 |
| SSAPC2P3 | 2251 | 2571 | 39 | 213 | 263 | 21 | 993 | 1027 | 11 | 107 | 138 | 15 | 527 | 537 | 6 | 91 | 120 | 18 |
| SSAPC3P1 | 3821 | 4333 | 47 | 817 | 855 | 14 | 1710 | 1785 | 16 | 96 | 130 | 17 | 815 | 832 | 8 | 122 | 130 | 6 |
| SSAPC3P2 | 3550 | 4158 | 54 | 578 | 608 | 12 | 1686 | 1758 | 16 | 100 | 125 | 13 | 710 | 733 | 11 | 68 | 73 | 4 |
| SSAPC3P3 | 2847 | 3336 | 48 | 416 | 642 | 121 | 1060 | 1144 | 21 | 129 | 154 | 13 | 491 | 520 | 13 | 124 | 125 | 2 |
| SSEFOR1 | 1320 | 1554 | 34 | 349 | 369 | 11 | 632 | 706 | 18 | 341 | 439 | 28 | 338 | 352 | 7 | 393 | 403 | 5 |
| SSEFOR2 | 2475 | 2839 | 39 | 241 | 252 | 7 | 883 | 1004 | 23 | 244 | 309 | 25 | 477 | 495 | 10 | 398 | 424 | 11 |
| SSEFOR3 | 3605 | 4137 | 49 | 295 | 317 | 10 | 773 | 948 | 32 | 258 | 303 | 18 | 641 | 674 | 15 | 424 | 448 | 10 |
| SSEARA1 | 3341 | 3945 | 54 | 349 | 362 | 7 | 1132 | 1225 | 20 | 490 | 596 | 29 | 774 | 796 | 9 | 681 | 733 | 15 |
| SSEARA2 | 3013 | 3544 | 54 | 492 | 519 | 12 | 1140 | 1238 | 21 | 260 | 333 | 27 | 455 | 470 | 8 | 614 | 653 | 14 |
| SSEARA3 | 3266 | 3829 | 53 | 402 | 463 | 22 | 1100 | 1204 | 21 | 481 | 525 | 14 | 503 | 546 | 18 | 823 | 871 | 14 |
| SSEPAS1 | 3179 | 3648 | 45 | 436 | 483 | 18 | 1258 | 1309 | 13 | 384 | 434 | 16 | 660 | 667 | 5 | 587 | 618 | 11 |
| SSEPAS2 | 3217 | 3773 | 53 | 616 | 667 | 15 | 1057 | 1124 | 16 | 251 | 312 | 21 | 310 | 336 | 15 | 257 | 273 | 8 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SSEPAS3 | 2933 | 3552 | 57 | 661 | 842 | 35 | 1367 | 1462 | 19 | 161 | 254 | 35 | 491 | 551 | 26 | 58 | 59 | 1 |

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**Table 3: Number of OTUs for the most representative insect orders.** The OTUs are separated by marker (18S and COI) and by type of sample (insects and soils). For bulk insect samples from 18S data, two other orders were well represented but almost absent in the other datasets: Orthoptera (145 OTUs) and Hemiptera (60 OTUs).

| | **18S** | | **COI** | |
|---|---|---|---|---|
| **Order** | **Soil** | **Insects** | **Soil** | **Insects** |
| Coleoptera | 32 | 179 | 157 | 71 |
| Diptera | 86 | 547 | 142 | 83 |
| Hymenoptera | 42 | 259 | 57 | 28 |
| Lepidoptera | 6 | 57 | 84 | 39 |
| Others | 120 | 340 | 155 | 67 |
| Total | 286 | 1382 | 595 | 288 |

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Figure 1 Sampling design within a specific locality. (A) Location of plots in one locality: three plots are set up in each major vegetation type present (illustrated here by different shades of green), (B) Scheme for one plot, showing the Malaise or Slam trap in the middle, and twenty trees arbitrarily chosen for soil sampling within a 28-m radius (in red circles), (C) The Malaise or Slam trap provides insects and the plants consumed by them, as well as any parasites or parasitoids in their bodies, while the soil samples provide eDNA for a large proportion of the habitat's total biodiversity (e. g. soil organisms, roots, leaves, dead animals, and faeces). Together, this framework covers a large proportion of the biodiversity in the plot, (D) Photo of a Slam trap used in Brazil, (E) Photo of a Malaise trap in a forest fragment in Sweden, (F) the Malaise trap used in the coastal area of South Africa.

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Figure 2. Map of sampling localities. The sampling localities used for this study cover a wide geographical range comprising the Amazonian rainforest in Brazil, the western coast of South Africa, and central-south Sweden. In each locality, different environments were sampled to test the usefulness of our methodology in a wide range of habitats. Habitats are: TF = terra-firmes, VZ = várzeas, and IG = igapós in Brazil, PC1, PC2, and PC2 in South Africa, and ARA = arable farms, FOR = forest fragments, and PAS = pasture farms in Sweden. The green gradient represent biomes described in Olson et al. (2001), ranging from densely forested areas (dark green) to open areas (light green).

Figure 3: Rarefaction curves. Rarefaction by sample for the A) the prokaryote dataset (16S), B) the eukaryote dataset for 18S, and C) the eukaryote dataset for COI. The red lines show the minimum number of reads. The 18S marker data are more variable in read number than are the 16S and COI data. In the COI data, all curves tend towards an asymptote.

Figure 4: Taxonomic composition of the OTU communities. These bar plots show the fraction of OTUs by taxonomic groups for each country for soil (black bars) and insect (gray bars) samples. There is no clear taxonomic variation among groups for country, but a clear difference by kind of samples, except for the COI datasets.

Figure 5: Community structure related to country and habitat type. Visualization of non-metric multidimensional scaling (NMDS) for 16S and 18S OTU communities. A) prokaryote (16S) colored by country, B) eukaryote (18S) OTU colored by country, C) prokaryote (16S) colored by country, and D) eukaryote (18S) colored by habitat type. Triangles represent soil samples and circles represent insect samples. Habitat abbreviations as in Fig. 2. These results show that the kind of sample (soil or insects) is the main explanatory factor underpinning similarity among localities. The clustering of communities per country is stronger than per habitat type.

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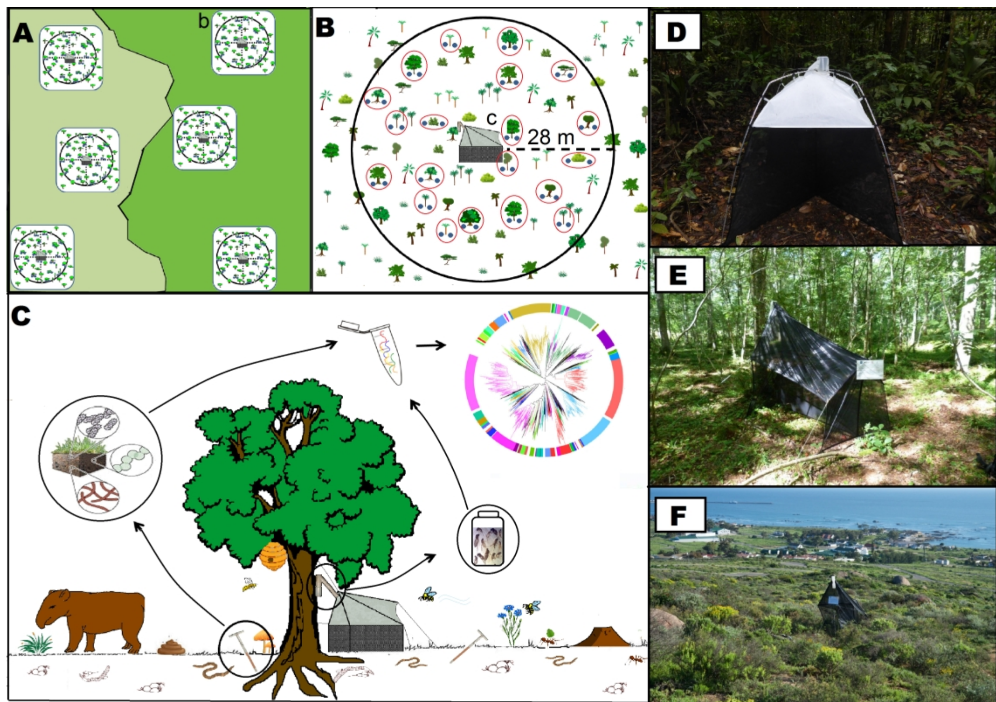
A
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28 m
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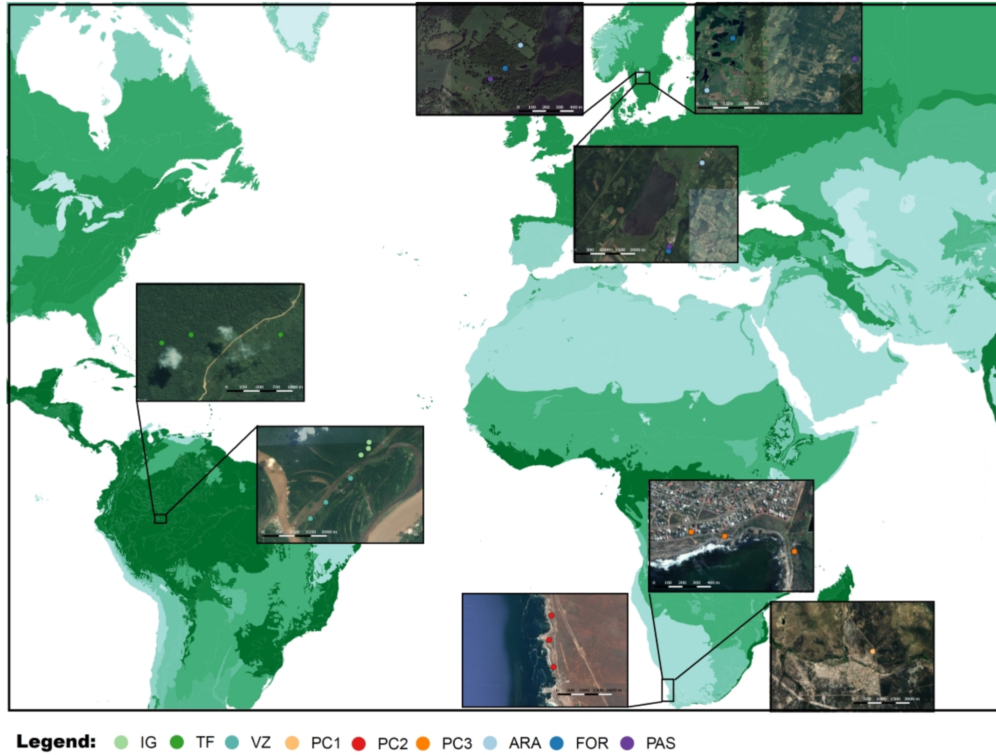
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IG
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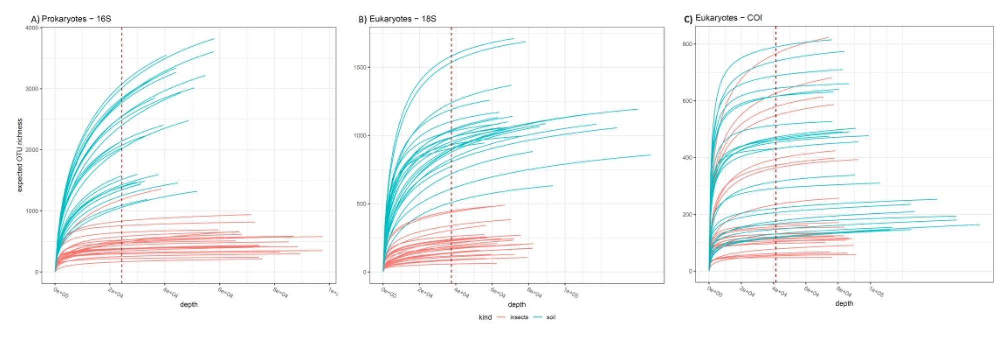
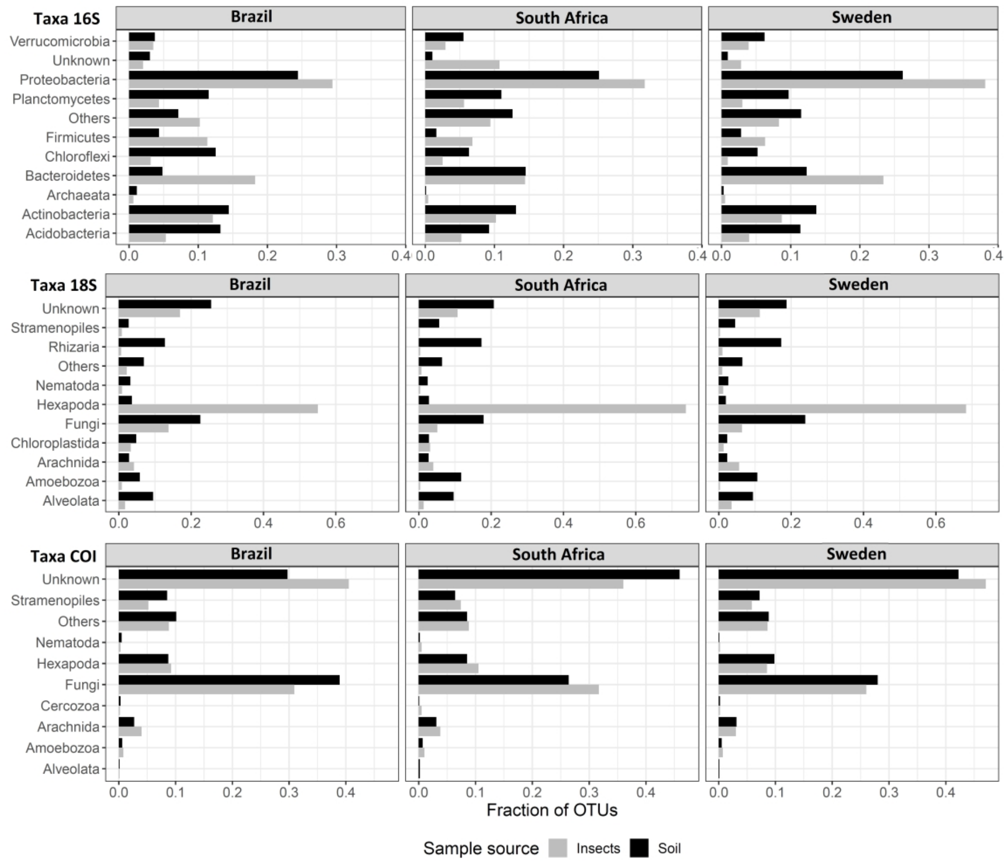
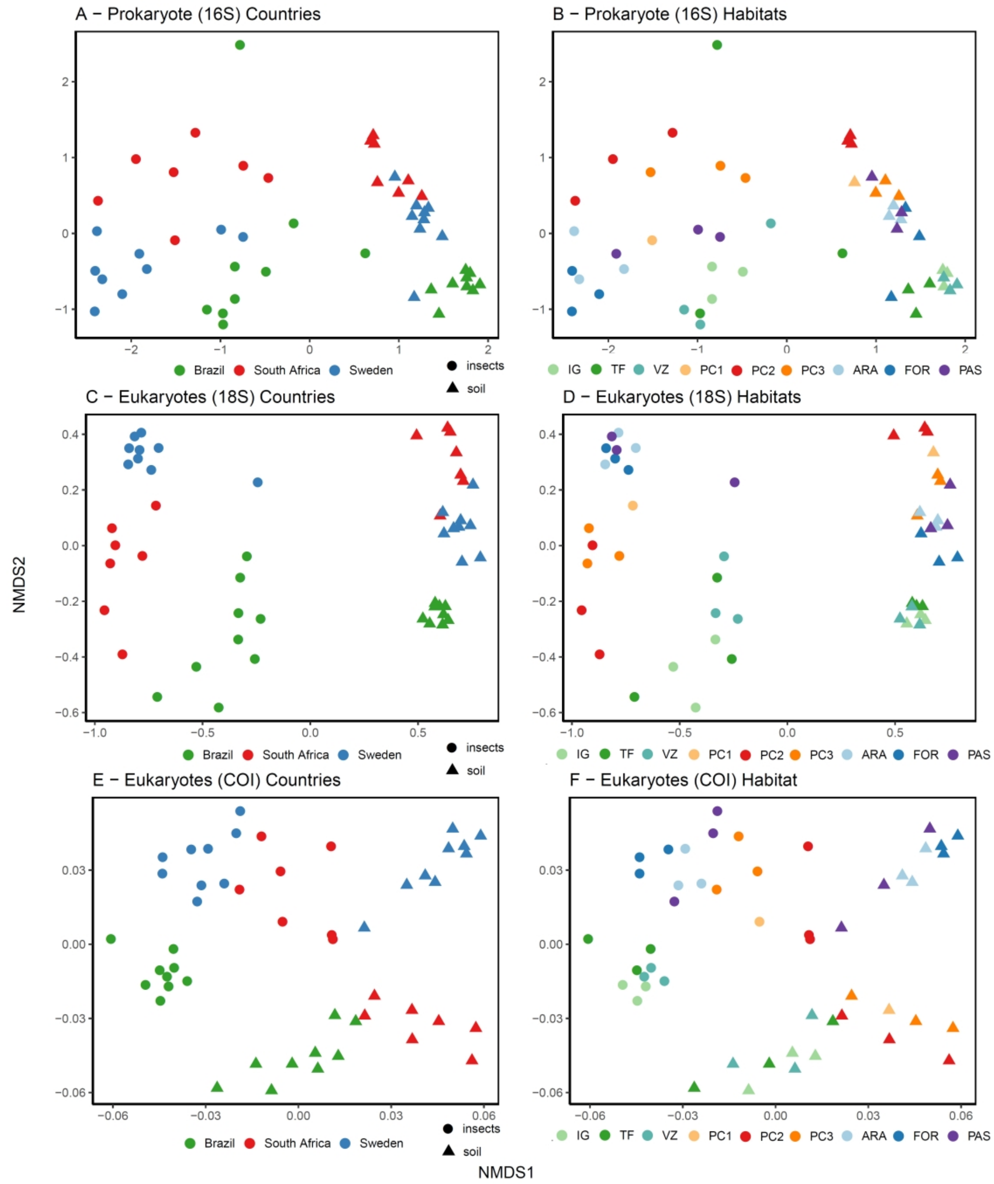

A) Prokaryotes − 16S
B) Eukaryotes − 18S
C) Eukaryotes − COI
expected OTU richness
0
1000
2000
3000
4000
500
1500
200
400
600
800
0e+00
2e+04
4e+04
6e+04
8e+04
1e+05
depth
kind
insects
soil


Taxa 16S
Brazil
South Africa
Sweden
Verrucomicrobia
Unknown
Proteobacteria
Planctomycetes
Others
Firmicutes
Chloroflexi
Bacteroidetes
Archaeata
Actinobacteria
Acidobacteria
Taxa 18S
Unknown
Stramenopiles
Rhizaria
Others
Nematoda
Hexapoda
Fungi
Chloroplastida
Arachnida
Amoebozoa
Alveolata
Taxa COI
Unknown
Stramenopiles
Others
Nematoda
Hexapoda
Fungi
Cercozoa
Arachnida
Amoebozoa
Alveolata
Fraction of OTUs
Sample source
Insects
Soil


A − Prokaryote (16S) Countries
B − Prokaryote (16S) Habitats
C − Eukaryotes (18S) Countries
D − Eukaryotes (18S) Habitats
E − Eukaryotes (COI) Countries
F − Eukaryotes (COI) Habitat
Brazil South Africa Sweden
insects
soil
IG TF VZ PC1 PC2 PC3 ARA FOR PAS
NMDS1
NMDS2