## Standardized NEON organismal data for biodiversity research

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- **Abstract**: Understanding patterns and drivers of species distributions and abundances, and thus biodiversity, is a core goal of ecology. Despite advances in recent decades, research into these patterns and processes is currently limited by a lack of standardized, high-quality, empirical data that spans large spatial scales and long time periods. The National Ecological Observatory Network (NEON) fills this gap by providing freely available observational data that are: generated during robust and consistent organismal sampling of several sentinel taxonomic groups within 81 sites distributed across the United States; available for at least 30 years. The breadth and scope of these data provides a unique resource for advancing biodiversity research. To maximize the potential of this opportunity, however, it is critical that NEON data be maximally accessible and easily integrated into investigators' workflows and analyses. To 43 facilitate its use for biodiversity research and synthesis, we created a workflows to process and format NEON organismal data into the ecocomDP (ecological community data design pattern) format, which we have made available in the ecocomDP R package; we then provided the standardized data as an R data package (neonDivData). We briefly summarize sampling designs 47 and data wrangling decisions for the major taxonomic groups included in this effort. Our workflows are open-source so the biodiversity community may: add additional taxonomic groups; modify the workflow to produce datasets appropriate for their own analytical needs; and regularly update the data packages as more observations become available. Finally, we provide 51 two simple examples of how the standardized data may be used for biodiversity research. By providing a standardized data package, we hope to enhance the utility of NEON organismal data
- Key words: NEON, Biodiversity, Organismal Data, Data Product, R, Data package

in advancing biodiversity research.

## Introduction (or why standardized NEON organismal data)

A central goal of ecology is to understand the patterns and processes of biodiversity, and this is particularly important in an era of rapid global environmental change (Midgley and Thuiller

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2005, Blowes et al. 2019). Such understanding is only possible through studies that address
   questions like: How is biodiversity distributed across large spatial scales, ranging from
   ecoregions to continents? What mechanisms drive spatial patterns of biodiversity? Are spatial
   patterns of biodiversity similar among different taxonomic groups, and if not, why do we see
   variation? How does community composition vary across spatial and environmental gradients?
   What are the local and landscape scale drivers of community structure? How and why do
   biodiversity patterns change over time? Answers to such questions will enable better
   management and conservation of biodiversity and ecosystem services.
   Biodiversity research has a long history (Worm and Tittensor 2018), beginning with major
   scientific expeditions (e.g., Alexander von Humboldt, Charles Darwin) aiming to document
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   global species lists after the establishment of Linnaeus's Systema Naturae (Linnaeus 1758).
   Beginning in the 1950's (Curtis 1959, Hutchinson 1959), researchers moved beyond
   documentation to focus on quantifying patterns of species diversity and describing mechanisms
   underlying their heterogeneity. Since the beginning of this line of research major theoretical
   breakthroughs (MacArthur and Wilson 1967, Hubbell 2001, Brown et al. 2004, Harte 2011) have
   advanced our understanding of potential mechanisms causing and maintaining biodiversity.
   Modern empirical studies, however, have been largely constrained to local or regional scales and
   focused on one or a few taxonomic groups, because of the considerable effort required to collect
   observational data. There are now unprecedented numbers of observations from independent
   small and short-term ecological studies. These data support research into generalities through
   syntheses and meta-analyses (Vellend et al. 2013, Blowes et al. 2019, Li et al. 2020), but this work
   is challenged by the difficulty of integrating data from different studies and with varying
   limitations. Such limitations include: differing collection methods (methodological
   uncertainties); varying levels of statistical robustness; inconsistent handling of missing data;
   spatial bias; publication bias; and design flaws (Martin et al. 2012, Nakagawa and Santos 2012,
   Koricheva and Gurevitch 2014, Welti et al. 2021). Additionally, it has historically been
   challenging for researchers to obtain and collate data from a diversity of sources, for use in
   syntheses and/or meta-analyses (Gurevitch and Hedges 1999).
   Barriers to meta-analyses have been remedied in recent years by large efforts to digitize museum
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and herbarium specimens (e.g., iDigBio), successful community science programs (e.g.,

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iNaturalist, eBird), technological advances (e.g., remote sensing, automated acoustic recorders),
   and long running coordinated research networks - which together bring biodiversity research
   into the big data era (Hampton et al. 2013, Farley et al. 2018). Yet, each of these comes with its
   own limitations. Museum/herbarium specimens and community science records are increasingly
   available, but are still incidental and unstructured in terms of the sampling design, and exhibit
   marked geographic and taxonomic biases (Martin et al. 2012, Beck et al. 2014, Geldmann et al.
   2016). Remote sensing approaches may cover large spatial scales, but may also be of low spatial
   resolution and unable to reliably penetrate vegetation canopy (Palumbo et al. 2017, G Pricope et
   al. 2019). The standardized observational sampling of woody trees by the United States Forest
   Service's Forest Inventory and Analysis and of birds by the United States Geological Survey's
   Breeding Bird Survey have been ongoing across the United States since 2001 and 1966,
   respectively (Bechtold and Patterson 2005, Sauer et al. 2017), but cover few taxonomic groups.
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   And while the Long Term Ecological Research Network (LTER) consists of 28 sites that provide
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   up to 40 years of observational and experimental datasets for a diverse set of ecosystems and
   taxa, there is no standardization in the design and data collections across sites (Jones et al. 2021).
   The same lack of standardized sampling is also true of the younger Critical Zone Observatory
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   (CZO) (Jones et al. 2021). Thus, despite recent advances biodiversity research is still impeded by
   a lack of standardized, high quality, and open-access data spanning large spatial scales and long
   time periods.
   The recently established National Ecological Observatory Network (NEON) provides
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   continental-scale observational and instrumentation data for a wide variety of taxonomic groups
   and measurement streams. Data are collected using standardized methods, across 81 field sites in
   both terrestrial and freshwater ecosystems, and will be freely available for at least 30 years.
   These consistently collected, long-term, and spatially robust measurements are directly
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   comparable throughout the Observatory, and provide a unique opportunity for enabling a better
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   understanding of ecosystem change and biodiversity patterns and processes across space and
   through time (Keller et al. 2008).
   NEON data are designed to be maximally useful to ecologists by aligning with FAIR principles
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   (findable, accessible, interoperable, and reusable Wilkinson et al. 2016). Despite meeting these
   requirements, however, there are still challenges to integrating NEON organismal data for
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reproducible biodiversity research. For example: field names may vary across NEON data products, even for similar measurements; some measurements include sampling unit 120 information, whereas units must be calculated for others; and data are in a raw form that often 12 includes metadata unnecessary for biodiversity analyses. These issues and inconsistencies may be overcome through data cleaning and formatting, but understanding how best to perform this task requires a significant investment in the comprehensive NEON documentation for each data 124 product involved in an analysis. Thoroughly reading large amounts of NEON documentation is 125 time consuming, and the path to a standard data format, as is critical for reproducibility, may vary greatly between NEON organismal data products and users - even for similar analyses. Ultimately, this may result in subtle differences from study to study that hinder meta-analyses 128 using NEON data. A simplified and standardized format for NEON organismal data would 129 facilitate wider usage of these datasets for biodiversity research. Furthermore, if these data were 130 formatted to interface well with datasets from other coordinated research networks, more 131 comprehensive syntheses could be accomplished and to advance macrosystem biology (Record et al. 2020). 133

One attractive standardized formatting style for NEON organismal data is that of ecocomDP 134 (ecological community data design pattern, O'Brien et al. In review). ecocomDP is the brainchild 135 of members of the LTER network, the Environmental Data Initiative (EDI), and NEON staff, and 136 provides a model by which data from a variety of sources may be easily transformed into 137 consistently formatted, analysis ready community-level organismal data packages. This is done 138 using reproducible code that maintains different dataset "levels" as data move through the model. Here we apply the "dataset level" terminology relative to our workflow, such that Lo in incoming data, L1 represents an intermediate data format, and L2 is the output format. For the purposes of describing this workflow, the NEON L1 data products that serve as inputs to our pipeline are 142 considered Level o (Lo) data in our workflow. In our workflow, Level 1 (L1) data are Lo data 143 transformed to the ecocomDP model and include tables representing observations, sampling locations, and taxonomic information (at a minimum). Finally, level 2 (L2) data represent data that have been further transformed for specific research studies. Thus far, >70 LTER organismal 146 datasets have been harmonized to the L1 ecocomDP format through the R package ecocomDP 147 and more datasets are in the queue for processing into the ecocomDP format by EDI. Note that

the levels used here are different from those used by NEON to describe their data processing levels (https://www.neonscience.org/data-samples/data-management/data-processing). We refer 150 to the input data streams provided by NEON as data products, whereas the cleaned and standardized collection of data files provided here as objects within an R data package, neonDivDate, across this paper. 153 Here, we briefly summarized NEON sampling designs for major taxonomic groups and major 154 data wrangling decisions we made to simplify and standardize NEON data products following the ecocomDP format using the R package ecocomDP. All corresponding R code to process NEON data products were included in the R package ecocomDP for transparency and so future 157 contributors may draw on this information in their work. To facilitate the distribution and usage 158 of the standardized datasets, we deposited them in the EDI data portal (link) and provided them 159 as an R data package neonDivData. Both of them will be maintained and updated as new data become available from the NEON portal. We hope this effort will substantially reduce data processing times for NEON data users and greatly facilitate the use of NEON organismal data to advance our understanding of Earth's biodiversity.

# Materials and Methods (or how to standardize NEON organismal data)

There are many details to consider when starting to use NEON organismal data products. Below 166 we outline key points relevant to community-level biodiversity analyses with regards to the 167 NEON Sampling Design and decisions that were made as the data products presented in this 168 paper were converted into the ecocomDP data model. While the methodological sections below are specific to particular taxonomic groups, there are some general points that we came across 170 while reviewing NEON data collection documentation and through discussion with NEON staff 171 that apply to all NEON organismal data products. First, species occurrence and abundance 172 measures as reported in NEON biodiversity data products are not standardized to sampling effort. Because there are often multiple approaches to cleaning (e.g., dealing with multiple levels of 174 taxonomic resolution, interpretations of absences, etc.) and standardizing biodiversity survey

Table 1: Mapping NEON data products to ecocomDP formatted data packages with abundances *standardized* to observation effort. \*BIRD counts are reported per taxon per "cluster" observed in each point count in the NEON data product and have not been further standardized to sampling effort because standard methods for modeling bird abundances are beyond the scope of this paper. \*\*Incidence rate per number of tests conducted is reported for TICK\_PATHOGENS.

Taxon group	Lo dataset (NEON L1 data product ID)	Lo to L1 ecocomDP workflow ID	Primary variable reported in ecocomDP observation table	Units
Algae	DP1.20166.001	neon.ecocomdp.20166.001.001	cell density OR cells OR valves	cells/cm2 OR cells/mL
Beetles	DP1.10022.001	neon.ecocomdp.10022.001.001	abundance	count per trap day
Birds	DP1.10003.001	neon.ecocomdp.10003.001.001	cluster size	count of individuals
Fish	DP1.20107.001	neon.ecocomdp.20107.001.001	abundance	catch per unit effort
Herptiles	DP1.10022.001	neon.ecocomdp.10022.001.002	abundance	count per trap day
Macroinvertebrates	DP1.20120.001	neon.ecocomdp.20120.001.001	density	count per square meter
Mosquitoes	DP1.10043.001	neon.ecocomdp.10043.001.001	abundance	count per trap hour
Plants	DP1.10058.001	neon.ecocomdp.10058.001.001	percent cover OR presence absence	percent of plot area covered by taxon OR Unitless
Small_mammals	DP1.10072.001	neon.ecocomdp.10072.001.001	count	unique individuals per 100 trap nights per plot per month
Ticks	DP1.10093.001	neon.ecocomdp.10093.001.001	abundance	count per square meter
Tick_pathogens	DP1.10092.001	neon.ecocomdp.10092.001.001	positivity rate	positive tests per pathogen per sampling event
Zooplankton	DP1.20219.001	neon.ecocomdp.20219.001.001	density	count per liter

data, NEON publishes raw observations along with sampling effort data to preserve as much information as possible so that data users can clean and standardize data as they see fit. The workflows described here for twelve taxonomic groups represented in eleven NEON data 178 products produce standardized counts based on sampling effort, such as count of individuals per 179 area sampled or count standardized to the duration of trap deployment, as described in Table 1. The data wrangling workflows described below can be used to access, download, and clean data 181 from the NEON Data Portal by using the RecocomDP package. To view a catalog of available NEON data products in the ecocomDP format, use ecocomDP::search\_data("NEON"). To 183 import data from a given NEON data product into your R environment, use 184 ecocomDP::read\_data(), and set the id argument to the selected NEON to ecocomDP mapping 186 workflow (the "Lo to L1 ecocomDP workflow ID" in Table 1). This will return a list of ecocomDP 186 formatted tables and accompanying metadata. To create a flat data table (similar to the L2 data packages described below), use the ecocomDP::flatten\_ecocomDP() function. Second, it should be noted that NEON data collection efforts will continue well after this paper is published and 189 new changes to data collection methods and/or processing may vary over time. Such changes (e.g., change in the number of traps used for ground beetle collection) or interruptions (e.g., due to COVID-19) to data collection are documented in the Issues log for each data product on the NEON Data Portal as well as the Readme text file that is included with NEON data downloads.

### **Terrestrial Organisms**

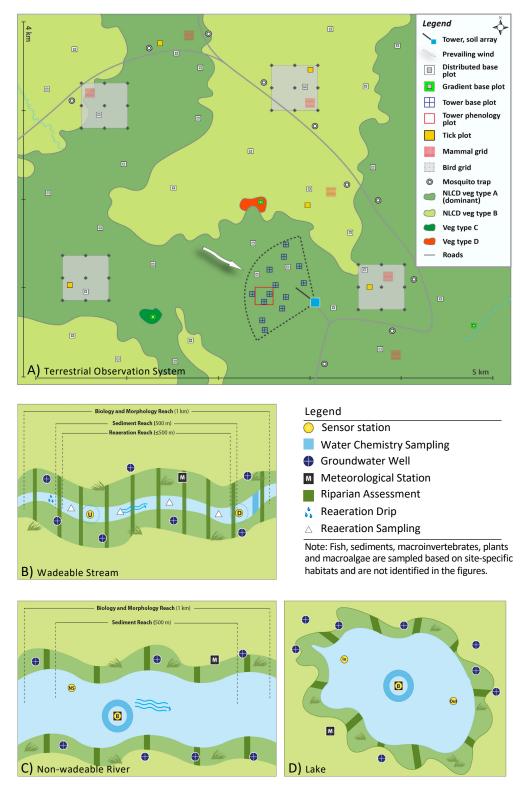


Figure 1: Generalized sampling schematics for Terrestrial Observation System (A) and Aquatic Observation System (B-D) plots. For Terrestrial Observation System (TOS) plots, Distributed, Tower, and Gradient plots, and locations of various sampling regimes, are presented via symbols. For Aquatic Observation System (AOS) plots, Wadeable streams, Non-wadeable streams, and Lake plots are shown in detail, with locations of sensors and different sampling regimes presented using symbols. Panel A was originally published in Thorpe et al. (2016).

### 195 Breeding Land Birds

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NEON Sampling Design NEON designates breeding landbirds as "smaller birds (usually
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   exclusive of raptors and upland game birds) not usually associated with aquatic habitats" (Ralph
   1993). Most species observed are diurnal and include both resident and migrant species.
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   Landbirds are surveyed via point counts in each of the 47 terrestrial sites. At most NEON sites,
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   breeding landbird points are located in five to ten 3 \times 3 m grids (Fig. 1), which are themselves
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   located in representative (dominant) vegetation. Whenever possible, grid centers are co-located
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   with distributed base plot centers. When sites are too small to support a minimum of five grids,
   separated by at least 250 m from edge to edge, point counts are completed at single points
   instead of grids. In these cases, points are located at the southwest corners of distributed base
   plots within the site. Five to 25 points may be surveyed depending on the size and spatial layout
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   of the site, with exact point locations dictated by a stratified-random spatial design that
   maintains a 250 m minimum separation between points.
   Surveys occur during one or two sampling bouts per season, at large and small sites respectively.
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   Observers go to the specified points early in the morning and track birds observed during each
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   minute of a 6-minute period, following a 2-minute acclimation period, at each point. Each point
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   count contains species, sex, and distance to each bird (measured with a laser rangefinder except
   in the case of flyovers) seen or heard. Information relevant for subsequent modeling of
   detectability is also collected during the point counts (e.g., weather, detection method). The point
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   count surveys for NEON were modified from the Integrated Monitoring in Bird Conservation
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   Regions (IMBCR) field protocol for spatially-balanced sampling of landbird populations
   (Pavlacky Jr et al. 2017).
   To protect species of concern, breeding landbird taxonomic IDs are 'fuzzed,' whereby the data are
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   provided with a taxonomic identification at one higher taxonomic level than where the
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   protection occurs. For example, if a threatened Black-capped vireo (Vireo atricapilla) is recorded
   by a NEON technician, the taxonomic identification is fuzzed to Vireo in the data. Rare,
   threatened and endangered species are those listed as such by federal and/or state agencies.
   Data Wrangling Decisions The bird point count data product ('DP1.10003.001') consists of a
   list of two associated data frames: brd countdata and brd perpoint. The former data frame
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contains information such as locations, species identities, and their counts. The latter data frame contains additional location information such as latitude and longitude coordinates and 225 environmental conditions during the time of the observations. The separate data frames are 226 linked by 'eventID,' which refers to the location, date and time of the observation. To prepare the 227 bird point count data for the L1 ecocomDP model, we first merged both data frames into one and then removed columns that are likely not needed for most community-level biodiversity analyses 220 (e.g., observer names, etc.). The field taxon\_id consists of the standard AOU 4-letter species code, although taxon\_rank refers to eight potential levels of identification (class, family, genus, species, speciesGroup, subfamily, and subspecies). Users can decide which level is appropriate, for example one might choose to exclude all unidentified birds (taxon\_id = UNBI), where no 233 further details are available below the class level (Aves sp.). The sampling protocol has evolved 234 over time, so users are advised to check whether the 'samplingProtocolVersion' associated with 235 bird point count data ('DP1.10003.001') fits their data requirements and subset as necessary. 236 Older versions of protocols can be found at the NEON document library.

#### 238 Ground Beetles and Herp Bycatch

NEON Sampling Design Ground beetle sampling is conducted via pitfall trapping, across 10 distributed plots at each NEON site. The original sampling design included the placement of a 240 pitfall trap at each of the cardinal directions along the distributed plot boundary, for a total of four traps per plot and 40 traps per site. In 2018, sampling was reduced via the elimination of the North pitfall trap in each plot, resulting in 30 traps per site. 243 Beetle pitfall trapping begins when the temperature has been >4°C for 10 days in the spring and 244 ends when temperatures dip below this threshold in the fall. Sampling occurs biweekly 245 throughout the sampling season with no single trap being sampled more frequently than every 12 days. After collection, the samples are separated into carabid species and bycatch. 247 Invertebrate bycatch is pooled to the plot level and archived. Vertebrate bycatch is sorted and 248 identified by NEON technicians, then archived at the trap level. Carabid samples are sorted and 240 identified by NEON technicians, after which a subset of carabid individuals are sent to be pinned and re-identified by an expert taxonomist. More details can be found in Hoekman et al. (2017).

Pitfall traps and sampling methods are designed by NEON to reduce vertebrate bycatch

(Hoekman et al. 2017). The pitfall cup is medium in size with a low clearance cover installed over

the trap entrance to minimize large vertebrate bycatch. When a live vertebrate with the ability to

move on its own volition is found in a trap, the animal is released. Live but morbund vertebrates

are euthanized and collected along with deceased vertebrates. When ≥15 individuals of a

vertebrate species are collected, cumulatively, within a single plot, NEON may initiate localized

mitigation measures such as temporarily deactivating traps and removing all traps from the site

for the remainder of the season. Thus, while herpetofaunal (herp) bycatch is present in many

pitfall samples it is unclear how well these pitfall traps capture herp community structure and

diversity - due to these active efforts to reduce vertebrate bycatch. Users of NEON herp bycatch

data should be aware of these limitations.

Data Wrangling Decisions The beetle and herp bycatch data product identifier is

'DDP1.10022.001.' Carabid samples are recorded and identified in a multi-step workflow wherein
a subset of samples are passed on in each successive step. Individuals are first identified by the
sorting technician after which a subset is sent on to be pinned. Some especially difficult
individuals are not identified by technicians during sorting, instead being labelled "other carabid."
The identifications for those individuals are recorded with the pinning data. Any individuals for
which identification is still uncertain are then verified by an expert taxonomist. There are a few
cases where an especially difficult identification was sent to multiple expert taxonomists and
they did not agree on a final taxon, these individuals were excluded from the data set at the
recommendation of NEON staff.

Preference is given to expert identification whenever available. However, these differences in taxonomic expertise do not seem to cause systematic biases in estimating species richness across sites, but non-expert taxonomists are more likely to misidentify non-native carabid species (Egli et al. 2020). Beetle abundances are recorded for the sorted samples by NEON technicians. To account for individuals who were later reidentified, the final abundance for a species is the original sorting sample abundance minus the number of individuals that were given a new ID.

Prior to 2018, trappingDays values were not included for many sites. Missing entries were calculated as the range from setDate through collectDate for each trap. We also accounted for a

few plots for which setDate was not updated based on a previous collection event in the trapping Days calculations. To facilitate easy manipulation of data within and across bouts a new 282 bout ID field was created to identify all trap collection events at a site in a bout. The original 283 EventID field is intended to identify a bout, but has a number of issues that necessitates creation 284 of a new ID. First, EventID does not correspond to a single collection date but rather all collections in a week. This is appropriate for the small number of instances when collections for 286 a bout happen over multiple consecutive days (~5% of bouts), but prevents analysis of bout 287 patterns at the temporal scale of a weekday. The data here were updated so all entries for a bout 288 correspond to the date (i.e., collectDate) on which the majority of traps are collected to maintain the weekday-level resolution with as high of fidelity as possible, while allowing for easy 290 aggregation within bouts and collectDate's. Second, there were a few instances in which plots 291 within a site were set and collected on the same day, but have different EventID's. These 202 instances were all considered a single bout by our new boutID, which is a unique combination of 293 setDate, collectDate, and siteID. Herpetofaunal bycatch (amphibian and reptile) in pitfall traps were identified to species or the 295 lowest taxonomic level possible within 24 h of recovery from the field. To process the herp bycatch NEON data we cleaned trapping Days and the other variables and added bout ID as described above for beetles. The variable sample Type in the bet\_sorting table provides the type of animal caught in a pitfall trap as one of five types: 'carabid,' 'vert bycatch herp,' 'other carabid,' 299 'invert bycatch' and 'vert bycatch mam.' We filtered the beetle data described above to only 300 include the 'carabid' and 'other carabid' types. For herps, we only kept the sample Type of 'vert 30 bycatch herp.' Abundance data of beetles and herps bycatch were standardized to be the number of individuals captured per trap day.

#### 4 Mosquitos

NEON Sampling Design Mosquito specimens are collected at 47 terrestrial sites across all
NEON domains and the data are reported in NEON data product DP1.10043.001. Traps are
distributed throughout each site according to a stratified-random spatial design used for all
Terrestrial Observation System sampling, maintaining stratification across dominant (>5% of

total cover) vegetation types. The number of mosquito traps placed in each vegetation type is proportional to its percent cover, until 10 total mosquito traps have been placed in the site. 310 Mosquito traps are typically located within 30 m of a road to facilitate expedient sampling, and are placed at least 300 m apart to maintain independence. Mosquito monitoring is divided into off-season and field season sampling. Off-season sampling 313 begins after three consecutive zero-catch field sampling bouts have occurred, and represents a 314 reduced sampling regime that is designed for the rapid detection of when the next field season should begin and to provide mosquito phenology data. Off-season sampling is conducted at three 316 dedicated mosquito traps spread throughout each core site, while temperatures are >10 °C. Once 317 per week, technicians deploy traps at dusk and then collect them at dawn the following day. 318 Field season sampling begins when the first mosquito is detected during off season sampling. Technicians deploy traps at all 10 dedicated mosquito trap locations per site. Traps remain out for a 24-hour period, or sampling bout, and bouts occur every two or four weeks at core and 32 relocatable terrestrial sites, respectively. During the sampling bout, traps are serviced twice and yield one night-active sample, collected at dawn or about eight hours after the trap was set, and one day-active sample, collected at dusk or ~16 hours after the trap was set. Thus, a 24-hour 324 sampling bout yields 20 samples from 10 traps. 325 NEON collects mosquito specimens using Center for Disease Control (CDC) CO<sub>2</sub> light traps. 326 These traps have been used by other public health and mosquito-control agencies for a half-century, so that NEON mosquito data align across NEON field sites and with existing long-term data sets. A CDC CO<sub>2</sub> light trap consists of a cylindrical insulated cooler that contains 329 dry ice, a plastic rain cover attached to a battery powered light/fan assembly, and a mesh 330 collection cup. During deployment, the dry ice sublimates and releases CO<sub>2</sub>. Mosquitoes 331 attracted to the CO2 bait are sucked into the mesh collection cup by the battery-powered fan, 332 where they remain alive until trap collection. 333 Following field collection, NEON's field ecologists process, package, and ship the samples to an 334 external lab where mosquitoes are identified to species and sex (when possible). A subset of identified mosquitoes are tested for infection by pathogens to quantify the presence/absence and prevalence of various arboviruses. Some mosquitoes are set aside for DNA barcode analysis as

well as long-term archiving. Particularly rare or difficult to identify mosquito specimens are prioritized for DNA barcoding. More details can be found in Hoekman et al. (2016). 339 Data Wrangling Decisions The mosquito data product (DP1.10043.001) consists of four data frames: trapping data (mos\_trapping), sorting data (mos\_sorting), archiving data 34 (mos\_archivepooling), and expert taxonomist processed data 342 (mos\_expertTaxonomistIDProcessed). We first removed rows (records) with missing 343 information about location, collection date, and sample or subsample ID for all data frames. We then merged all four data frames into one, wherein we only kept records for target taxa (i.e., targetTaxaPresent = "Y") with no known compromised sampling condition (i.e., sampleCondition 346 = "No known compromise"). We further removed a small number of records with species 347 identified only to the family level; all remaining records were identified at least to the genus level. 348 We estimated the total individual count per trap-hour for each species within a trap as (individualCount/subsampleWeight) \* totalWeight / trapHours. We then removed columns 359 that were not likely to be used for calculating biodiversity values. 35

#### Small Mammals

NEON Sampling Design NEON defines small mammals based on taxonomic, behavioral, dietary, and size constraints, and includes any rodent that is (1) nonvolant; (2) nocturnally active; 354 (3) forages predominantly aboveground; and (4) has a mass >5 grams, but <~ 500-600 grams. In North America, this includes cricetids, heteromyids, small sciurids, and introduced murids, but excludes shrews, large squirrels, rabbits, or weasels, although individuals of these species may be 357 incidentally captured. Small mammals are collected at NEON sites using Sherman traps, 358 identified to species in the field, marked with a unique tag, and released. Multiple 90 m  $\times$  90 m 359 trapping grids are set up in each terrestrial field site within the dominant vegetation type. Each 90 m  $\times$  90 m trapping grid contains 100 traps placed in a pattern with 10 rows and 10 columns 36 set 10 m apart. Three 90 m  $\times$  90 m grids per site are designated pathogen grids and the 362 remainder are designated diversity grids. Small mammal sampling occurs in bouts, with a bout 363 comprised of three consecutive (or nearly consecutive) nights of trapping at each pathogen grid and one night of trapping at each diversity grid. The timing of sampling occurs within 10 days

before or after the new moon. The number of bouts per year is determined by site type: core sites are typically trapped for 6 bouts per year (except for areas with shorter seasons due to cold weather), while relocatable sites are trapped for 4 bouts per year.

Data Wrangling Decisions In the small mammal data product (DP1.10072.001), records are stratified by NEON site, year, month, and day and represent data from both the diversity and pathogen sampling grids. Capture records were removed if they were not identified to genus or species (e.g., if the species name was denoted as 'either/or' or as family name), if their trap status is not "5 - capture" or "4 - more than 1 capture in one trap." Abundance data for each plot and month combination were standardized to be the number of individuals captured per 100 trap nights.

#### Soil Microbes

**NEON Sampling Design** Soil samples are collected at ten 40 m  $\times$  40 m NEON plots per site. Four plots are within the tower airshed (tower plots), and six plots are distributed across the landscape (gradient plots). At each sampling time, soils are sampled from three of four subplots, and one sample is collected from a randomly-generated XY coordinate location within each 380 subplot. At each sampling location, soils are taken at the surface horizon most years, but from 38 both organic and mineral horizons every five years during coordinated microbe/biogeochemistry bouts. Most sites, except for the boreal/arctic sites, are sampled three times a year, once at peak vegetation greenness and two other times bracketing that period. This results in  $\sim$ 10 plots  $\times$  3 locations  $\times$  1 or 2 horizons  $\times$  3 periods = 90 - 180 soil samples per site per year for most sites. 385 Samples for microbial biomass, composition, and metagenomics are stored on dry ice and 386 shipped to an external lab (variable depending on year) for downstream processing. 387 Data Wrangling Decisions Unlike other NEON biodiversity data products, the soil microbial data product (DP1.10107.001) requires significant pre-processing to go from raw sequence data 389 to a community matrix, and the exact bioinformatics methods will vary depending on use case. Briefly, major decisions during this process will depend on whether users are working with fungal (ITS) or bacterial (16S) data, if the goal is to maximize read quality and taxonomic resolution vs. number of reads retained through the quality filter process, and whether to remove

bioinformatics pipeline, how to run sensitivity analyses on user-defined parameters, 395 accompanying code, and vignettes are described in Qin et al. in this issue. At the end of the suggested bioinformatics pipeline, users will have a phyloseq object, which is a commonly-used format for sequence-based analysis software. The phyloseq object will contain a table of ASV 398 (amplicon sequence variant) sequences, a table of taxonomic assignments, and soil chemical and 399 physical data associated with corresponding sample locations and sampling bouts. Note that NEON provides both ITS and 16S marker gene sequence data for soil, benthic, and 40 surface water microbes in data products DP1.10108.001, DP1.20280.001, and DP1.20282.001, 402 respectively. Based on the input from the potential data user community, NEON staff scientists 403 have recently developed an in-house bioinformatics pipeline to produce associated derived soil, 404 benthic, and surface water microbe community composition data products, which are published as DP1.10081.001, DP1.20086.001, and DP1.20141.001, respectively. However, at the time of writing this, the completed community composition data products have not been fully published; 407 thus, they focus on the soil microbial marker gene sequences data product (DP1.10108.001), 408 which has the most extensive spatial and temporal coverage for data availability out of the marker gene sequence data products.

or retain reverse complement reads for a merged sequence. The full description of a suggested

#### 411 Terrestrial Plants

NEON Sampling Design NEON plant diversity sampling is completed once or twice per year (one or two 'bouts') in multiscale, 20 m × 20 m (400 m²) plots. The multiscale plot design subdivides into four 10 m × 10 m (100 m²) subplots that each encompass one or two sets of 3.16 m × 3.16 m (10 m²) subplots within which a 1 m × 1 m (1 m²) subplot is nested. The cover of each species is estimated visually in the 1 m² subplots. The presence of species is documented in the 10 m² and 100 m² subplots, when aggregated these data represent incidence of species at the 400 m² plot scale. In practice in the field, cover by species is recorded in a 1 m² subplot. Next, the remaining 9 m² area of the associated 10 m² subplot is searched for the presence of species. The process is repeated if there is a second 1 and 10 m² nested pair in the specific 100 m² subplot.

Next, the remaining 80 m² area is searched for the presence of species; data can be aggregated for

a complete list of species present at the 100 m<sup>2</sup> subplot scale. In most cases, species encountered in a 10 m<sup>2</sup> subplot that were already documented in the nested 1 m<sup>2</sup> subplot are not recorded as 423 the 1 m<sup>2</sup> subplot occupies 1 m<sup>2</sup> of the associated 10 m<sup>2</sup> subplot. The same is true of species reported in the 100 m² subplots, only species not yet reported in the nested 1 and 10 m² subplot(s) are reported. A full dataset for each NEON plant diversity plot was generated by combining all data from all subplots within the 20 m × 20 m plot, and removing duplicates 427 (which may occur when recording and/or aggregating species presence across nested subplots within each 100 m<sup>2</sup> subplot and across the four 100 m<sup>2</sup> subplots). More details about the sampling design can be found in Barnett et al. (2019). NEON manages plant taxonomic entries with a master taxonomy list that is based on the 431 community standard, where possible. Using this list, synonyms for a given species are converted 432 to the currently used name. The master taxonomy for plants is the USDA PLANTS Database (USDA, NRCS. 2014. https://plants.usda.gov), and the portions of this database included in the 434 NEON plant master taxonomy list are those pertaining to native and naturalized plants present 435 within the NEON sampling area. A sublist for each NEON domain includes those species with

ranges that overlap the domain as well as nativity designations - introduced or native - in that
part of the range. If a species is reported at a location outside of its known range, and the record
proves reliable, the master taxonomy list is updated to reflect the distribution change. For more

on the NEON plant master taxonomy list see NEON.DOC.014042

(https://data.neonscience.org/api/vo/documents/NEON.DOC.014042vK).

**Data Wrangling Decisions** In the plant presence and percent cover data product (DP1.10058.001) sampling at the 1 m  $\times$  1 m scale also includes observations of abiotic and 443 non-target species ground cover (i.e., soil, water, downed wood), so we removed records with 444 divDataType as "otherVariables." We also removed records whose targetTaxaPresent is N (i.e., 445 a non-target species). Additionally, for all spatial resolutions (i.e., 1 m<sup>2</sup>, 10 m<sup>2</sup>, and 100 m<sup>2</sup> data), any record lacking information critical for combining data within a plot and for a given sampling 447 bout (i.e., plotID, subplotID, boutNumber, endDate, or taxonID) was dropped from the dataset. 448 Furthermore, records without a definitive genus or species level taxonID (i.e., those representing unidentified morphospecies) were not included. To combine data from different spatial 450 resolutions into one data frame, we created a pivot column entitled sample area m2 (with

possible values of 1, 10, and 100). Because of the nested sampling design of the plant data, to
capture all records within a subplot at the 100 m<sup>2</sup> scale, we incorporated all data from both the 1
m<sup>2</sup> and 10 m<sup>2</sup> scales for that subplot. Similarly, to obtain all records within a plot at the 400 m<sup>2</sup>
scale, we included all data from that plot. Species abundance information was only recorded as
area coverage within 1 m by 1 m subplots; however, users may use the frequency of a species
across subplots within a plot or plots within a site as a proxy of its abundance if needed.

#### Ticks and Tick Pathogens

NEON Sampling Design Tick sampling occurs in six distributed plots at each site, which are randomly chosen in proportion to NLCD land cover class. Sampling begins on a low intensity schedule with one sampling bout every six weeks. Once >5 ticks of any life stage have been collected within the last year at that site, sampling switches to a high intensity schedule with one 462 bout every three weeks. A site remains on the high intensity schedule until <5 ticks are collected 463 within a year, then it reverts back to the low intensity schedule. High intensity sampling 464 reoccurs when >5 ticks are collected within a year. Onset of sampling coincides with phenological milestones at each site, beginning within two weeks of the onset of green-up and ending within two weeks of vegetation senescence. Sampling bouts are only initiated if the high 467 temperature on the two consecutive days prior to planned sampling was >o°C. 468 Ticks are sampled by walking the perimeter of a 40 m  $\times$  40 m plot using a 1 m  $\times$  1 m drag cloth. Ideally, 160 meters are sampled (shortest straight line distance between corners), but the cloth can be dragged around obstacles if a straight line is not possible. Acceptable total sampling area 471 is between 80-180 meters. The cloth can also be flagged over vegetation when the cloth cannot be 472 dragged across it. Ticks are collected from the cloth and technicians' clothing at appropriate 473 intervals depending on vegetation density and at every corner of the plot and are immediately transferred to a vial containing 95% ethanol. 475 Ticks are sent to the US National Tick Collection at Georgia Southern University for 476 identification to species, life stage, and sex. A subset of nymphal ticks are sent to the Laboratory of Medical Zoology at the University of Massachusetts Amherst for pathogen testing. Ixodes scapularis are tested for Anaplasma phagocytophilum, Babesia microti, Borrelia burgdorferi sensu

lato, Borrelia miyamotoi, Borrelia mayonii, other Borrelia species (Borrelia sp.), and a Ehrlichia muris-like agent (Pritt et al. 2017). Amblyomma americanum was tested for Anaplasma 48 phagocytophilum, Borrelia lonestari (and other undefined Borrelia species), Ehrlichia chaffeensis, Ehrlichia ewingii, Francisella tularensis, and Rickettsia rickettsii. **Data Wrangling Decisions** The tick data product (DP1.10093.001) consists of two dataframes: 484 'tck\_taxonomyProcessed' hereafter referred to as 'taxonomy data' and 'tck\_fielddata' hereafter referred to as 'field data.' Users should be aware of some issues related to taxonomic ID. Counts 486 assigned to higher taxonomic levels (e.g., at the order level *Ixodida*; IXOSP2) are not the sum of lower levels; rather they represent the counts of individuals that could not reliably be assigned to 488 a lower taxonomic unit. Samples that were not identified in the lab were assigned to the highest 489 taxonomic level (order Ixodida; IXOSP2). However, users could make an informed decision to 490 assign these ticks to the most probable group if a subset of individuals from the same sample were assigned to a lower taxonomy. 492 To clean the tick data, we first removed surveys and samples not meeting quality standards. In 493 the taxonomy data, we removed samples where sample condition was not listed as "OK" (<1% of records). In the field data, we removed records where samples were not collected due to logistical 495 concerns (10%). We then combined male and female counts in the taxonomy table into one 496 "adult" class. The taxonomy table was re-formatted so that every row contained a sampleID and 497 counts for each species life-stages were separate columns (i.e., "wide format"). Next, we joined 498 the field data to the taxonomy data, using the sample ID to link the two tables. When joining, we retained field records where no ticks were found in the field and thus there were no associated taxonomy data. In drags where ticks were not found, counts were given zeros. All counts were 50 standardized by area sampled. 502 Prior to 2019, both field surveyors and laboratory taxonomists enumerated each tick life-stage; consequently, in the joined dataset there were two sets of counts ("field counts" and "lab counts"). 504 However, starting in 2019, counts were performed by taxonomists rather than field surveyors. Field surveys conducted after 2019 no longer have field counts. Users of tick abundance data 506 should be aware that this change in protocol has several implications for data wrangling and for

analysis. First, after 2019, tick counts are no longer published at the same time as field survey

data. Subsequently, some field records from the most recent years have tick presence recorded (targetTaxaPresent = "Y"), but do not yet have associated counts or taxonomic information and 510 so the counts are still listed as NA. We have added a column to designate whether counts are still pending as of this data release. Users should be aware that counts of zero are therefore published earlier than positive counts. We strongly urge users to filter data to those years where there are 513 no counts pending. 514 The second major issue is that in years where both field counts and lab counts were available, they did not always agree (8% of records). In cases of disagreement, we generally used lab counts 516 in the final abundance data, because this is the source of all tick count data after 2019 and 517 because life-stage identification was more accurate. However, there were a few exceptions where 518 we used field count data. In some cases, only a subsample of a certain life-stage was counted in the lab, which resulted in higher field counts than lab counts. In this case, we assigned the additional un-identified individuals (e.g., the difference between the field and lab counts) to the 52 order level (IXOSP2). If quality notes from NEON described ticks being lost in transit, we also 522 added the additional lost individuals to the order level. There were some cases (<1%) where the field counts were greater than lab counts by more than 20% and where the explanation was not obvious; we removed these records. We note that the majority of samples (~85%) had no discrepancies between the lab or field, therefore this process could be ignored by users whose 526 analyses are not sensitive to exact counts. 527 The tick pathogen data product (DP1.10092.001) consists of two dataframes: tck pathogen hereafter referred to as 'pathogen data' and tck pathogen a hereafter referred to as 'quality data.' First, we removed any samples that had flagged quality checks from the quality data and 530 removed any samples that did not have a positive DNA quality check from the pathogen data. 53 Although the original online protocol aimed to test 130 ticks per site per year from multiple tick species, the final sampling decision was to extensively sample IXOSCA, AMBAME, and AMBSP species only because IXOPAC and *Dermacentor* nymph frequencies were too rare to generate 534 meaningful pathogen data. Borrelia burgdorferi and Borrelia burgdorferi sensu lato tests were 535 merged, since the former was an incomplete pathogen name and refers to B. burgdorferi sensu 536 lato as opposed to sensu stricto (Rudenko et al. 2011). Tick pathogen data are presented as 537

positivity rate calculated as number positive tests per number of tests conducted for a given

pathogen on ticks collected during a given sampling event.

### 40 Aquatic Organisms

#### Aquatic macroinvertebrates

NEON Sampling Design Aquatic macroinvertebrate sampling occurs three times/year at wadeable stream, river, and lake sites from spring through fall. Samplers vary by habitat and 543 include Surber, Hess, hand corer, modified kicknet, D-frame sweep, and petite ponar samplers. 544 Lake sampling occurs with a petite ponar near buoy, inlet, and outlet sensors, and D-frame 545 sweeps in littoral zones. Riverine sample collections in deep waters or near buoys are made with a petite ponar, and in littoral areas are made with a D-frame sweep or large-woody debris sampler. In the field, samples are preserved in pure ethanol, and later in the domain support facility, glycerol is added to prevent the samples from becoming brittle. Samples are shipped from the domain facility to a taxonomy lab for sorting and identification to lowest possible taxon (e.g., genus or species) and counts of each taxon per size are made to the nearest mm. Data Wrangling Decisions Aquatic macroinvertebrate data contained in the data product 552 DP1.20120.001 are subsampled and identified to the lowest practical taxonomic level, typically 553 genus, by expert taxonomists in the inv taxonomyProcessed table, measured to the nearest mm 554 size class, and counted. Taxonomic naming has been standardized in the inv taxonomyProcessed file, according to NEON's master taxonomy (https://data.neonscience.org/taxonomic-lists), removing any synonyms. We calculated 557 macroinvertebrate density by dividing estimated Total Count (which includes the corrections for 558 subsampling in the taxonomy lab) by benthicArea from the inv\_fieldData table to return count per square meter of stream, lake, or river bottom.

#### 561 MicroAlgae (Periphyton and Phytoplankton)

NEON Sampling Design Algal sampling methods vary by system (i.e., wadeable streams, rivers, lakes) and are sampled three times per year (i.e., spring, summer, and fall). In wadeable streams, which have variable habitats (e.g., riffles, runs, pools, step pools), five periphyton samples are

collected in the dominant habitat type and three in the second most dominant habitat type over a 1 km reach. No two samples should come from the sample habitat unit (i.e., the same riffle). 566 Periphyton samples are collected from natural surface substrata with the specific collection method and sampler type dependent on the substrate type (i.e., cobble vs. silt vs. woody debris) (see Moulton II et al. 2002 for detailed methods). Periphyton are also collected from lakes and rivers from three areas (five from 2014-2018) in the littoral zone (i.e., shoreline) with the most 570 dominant substratum type selected. In rivers, phytoplankton are sampled near the sensor buoy and at two other deep-water points in the main channel, using a Kemmerer sampler. For lakes, 572 phytoplankton are collected by the central sensor buoy as well as at the two littoral sensors, 573 using a Kemmerer sampler. For stratified lakes and non-wadeable streams, the phytoplankton 574 sample is a composite from one surface sample, one sample from the metalimnion (i.e., middle 575 layer), and one sample from the bottom of the euphotic zone. For non-stratified lakes and non-wadeable streams, the phytoplankton sample is a composite from one surface sample, one 577 sample just above the bottom of the euphotic zone, and if the euphotic zone is > 5 m, then one 578 mid-euphotic zone sample. 579 Samples are processed at the domain support facility and separated into subsamples for taxonomic analysis or for biomass measurements. Aliquots shipped to an external facility for

taxonomic analysis or for biomass measurements. Aliquots shipped to an external facility for taxonomic determination are preserved in glutaraldehyde or Lugol's iodine (before 2021).

Aliquots for biomass measurements are filtered onto glass-fiber filters and processed for ash-free dry mass.

Data Wrangling Decisions The periphyton, seston, and phytoplankton data product (DP1.20166.001) contains three dataframes for algae containing information on algae taxonomic identification, biomass and related field data, which are hereafter referred to as alg\_tax\_long, alg\_biomass and alg\_field\_data. Algae within samples are identified to the lowest possible taxonomic resolution, usually species, by contracting laboratory taxonomists. Some specimens can only be identified to the genus or even class level, depending on the condition of the specimen. Ten percent of all samples are checked by a second taxonomist and are noted in the qcTaxonomyStatus. Taxonomic naming has been standardized in the alg\_tax\_long files, according to NEON's master taxonomy, removing nomenclatural synonyms. Abundance and

cell/colony counts are determined for each taxon of each sample with counts of cells or colonies that are either corrected for sample volume or not (as indicated by algalParameterUnit = 595 cellsperBottle'). We corrected sample units of cellsperBottle to density. First, we summed the preservative 597 volume and the lab's recorded sample volume for each sample (from the alg\_biomass file) and 598 combined that with the alg\_tax\_long file using sampleID as a common identifier. Where 599 samples in the alg\_tax\_long file were missing data in the perBottleSampleVolume field (measured after receiving samples at the external laboratory), we estimated the sample volume using NEON domain lab sample volumes (measured prior to shipping samples to the external 602 laboratory). With this updated file, we combined it with alg\_field\_data to have the related field 603 conditions, including benthic area sampled for each sample. parentSampleID was used for 604 alg field data to join to the alg biomass file's sampleID as alg field data only has parentSampleID. We then calculated cells per milliliter for the uncorrected taxon of each sample, dividing algalParameterValue by the updated sample volume. Benthic sample results are expressed in terms of area (i.e., multiplied by the field sample volume, divided by benthic area 608 sampled), in square meters. The final abundance units are either cells/mL (phytoplankton and 600 seston samples) or cells/m² for benthic samples. The sampleIDs are child records of each parentSampleID that will be collected as long as 611 sampling is not impeded (i.e., ice covered or dry). In the alg biomass file, there should be only a 612 single entry for each parentSampleID, sampleID, and analysisType. Most often, there were two 613 sampleID's per parentSampleID with one for ash-free dry mass (AFDM) and taxonomy (analysis types). For the creation of the observation table with standardized counts, we used only records from the alg\_biomass file with the analysisType of taxonomy. In alg\_tax\_long, there 616 are multiple entries for each sampleID for each taxon by scientificName and algalParameter.

#### 8 Fish

NEON Sampling Design Fish sampling is carried out across 19 of the NEON eco-climatic
domains including both lotic (23 stream habitats) and lentic (five lake habitats) habitats. Each site
is surveyed with two bouts per year, during the spring and fall, with a combination of random

and fixed sampling areas (i.e., segments in lakes; reaches in streams) to capture spatial and temporal heterogeneity of aquatic habitats. Each sampling bout is completed within five days 623 with a minimum two-week gap in between two successive sampling bouts. The initial sampling 624 date is determined using site-specific historical data on ice melting, water temperature (or 625 accumulated degree days), and riparian peak greenness. At each lake, 10 pie-shaped segments are established, wherein each segment ranges from the 627 riparian zone into the lake center, therefore effectively capturing both nearshore and offshore 628 habitats. Three of the 10 segments are fixed and are surveyed twice a year with a backpack electrofisher using a three-pass electrofishing depletion approach (Moulton II et al. 2002, Peck et 630 al. 2006). All three passes in a fixed sampling segment or reach are completed on the same night, 631 with ≤30-minutes between successive passes. Electrofishing is started and ceases 30-minutes 632 after and before sunset and sunrise, respectively, with a maximum of five passes per sampling bout. Gill nets are deployed for 1-2 hrs either in the morning or early afternoon. Additionally, a fyke net and a gill net are deployed at each fixed segment (Baker et al. 1997). Fyke nets are 635 positioned before sunset and recovered after sunrise on the following day. The remaining 636 random segments are sampled on a rotation design with a single electrofishing pass, one mini-fyke net, and one gill net where three segments are surveyed twice a year. Precise start and end times for both electrofishing and net deployments are documented. 639 In each stream site, a maximum of 10 non-overlapping reaches (where each reach is 70-130 m in length) are designated within a 1 km stream length, and six of those reaches are sampled during any given sampling bout. The 10 reaches include three fixed reaches that are consistently sampled and seven random reaches that are sampled on rotation. The three fixed reaches 643 encompass all representative habitats found within a 1 km stretch and are surveyed twice a year 644 (once in each bout) using a three-pass electrofishing depletion approach (Moulton II et al. 2002, 645 Peck et al. 2006). The random reaches are surveyed on rotation via a single-pass depletion approach; three random reaches are sampled twice a year (once in each bout), while another three random reaches are sampled in the subsequent year. Electrofishing at streams is done 648 during daytime. 649

In all surveys, captured fish are identified to the lowest practical taxonomic level, and

morphometrics (i.e., body mass and body length) are recorded for 50 individuals of each taxon before releasing. Relative abundance for each fish taxon is also recorded by direct enumeration 652 (up to first 50 individuals) or estimation by bulk counts (>50 individuals, i.e., by placing fish of a 653 given taxon into a dip net (i.e., net scoop), counting the total number of specimens in the dip net, and then multiplying the total number of scoops of captured fish by the counts from the first 655 scoop). 656 Data Wrangling Decisions Fish sampled via both electrofishing and trapping are identified at variable taxonomic resolutions (as fine as subspecies level) in the field. Most identifications are made to the species or genus level by a single field technician for a given bout per site. Sampled 659 fish are identified, measured, weighed, and then released back to the site of capture. If field 660 technicians are unable to identify to the species level, such specimens are identified to the finest 661 possible taxonomic resolution or assigned a morphospecies with a coarse-resolution 662 identification. The standard sources consulted for identification and a qualifier for identification 663 validity are also documented in the fsh\_perFish table. The column bulkFishCount of the fsh\_bulkCount table records relative abundance for each species or the alternative next possible 665 taxon level (specified in the column scientificName). 666 Fish data (taxonomic identification and relative abundance) are recorded per each sampling reach in streams or per segment in lakes in each bout and documented in the fsh perFsh table. The 668 column eventID uniquely identifies the sampling date of the year, the specific site within the 669 domain, a reach/segment identifier, the pass number (i.e., number of electrofishing passes or 670 number of net deployment efforts), and the survey method. The eventID column helps tie all fish data with stream reach/lake segment data or environmental data (i.e., water quality data) and sampling effort data (e.g., electrofishing and net set time). A reachID column provided in the 673 fsh\_perPass table uniquely identifies surveys done per stream reach or lake segment. The 674 reachID is nested within the eventID as well. We used eventID as a nominal variable to uniquely identify different sampling events and to join different, stacked fish data files as described below. The fish data product (DP1.20107.001) consists of fsh\_perPass, fsh\_fieldData, fsh\_bulkCount, 677 fsh\_perFish, and the complete taxon table for fish, for both stream and lake sites. To join all 678 reach-scale data, we first joined the fsh\_perPass with fsh\_fieldData, and eliminated all bouts

where sampling was untenable. Subsequently, we joined the reach-scale table with fsh perFsh to add individual fish counts and fish measurements. Then, to add bulk counts, we joined the 68 reach-scale table with fsh\_bulkCount datasets, and subsequently added taxonRank which 682 included the taxonomic resolution into the bulk-processed table. Afterward, both individual-level 683 and bulk-processed datasets were appended into a single table. To include samples where no fish were captured, we filtered the fsh\_perPass table retaining records where target taxa (fish) were 685 absent, joined it with fsh\_fieldData, and finally merged it with the table that contained both 686 bulk-processed and individual-level data. For each finer-resolution taxon in the individual-level 68dataset, we considered the relative abundance as one since each row represented a single individual fish. Whenever possible, we substituted missing data by cross-referencing other data columns, omitted completely redundant data columns, and retained records with genus- and 690 species-level taxonomic resolution. For the appended dataset, we also calculated the relative 60 abundance for each species per sampling reach or segment at a given site. To calculate 602 species-specific catch per unit effort (CPUE), we normalized the relative abundance by either average electrofishing time (i.e., efTime, efTime2) or trap deployment time (i.e., the difference between netEndTime and netSetTime). For trap data, we assumed that size of the traps used, 695 water depths, number of netters used, and the reach lengths (a significant proportion of bouts 696 had reach lengths missing) to be comparable across different sampling reaches and segments.

#### Zooplankton

NEON Sampling Design Zooplankton samples are collected at seven NEON lake sites across four domains. Zooplankton samples are collected at the buoy sensor set (deepest location in the lake) and at the two nearshore sensor sets using a vertical tow net for locations deeper than 4 m and a Schindler trap for locations shallower than 4 m. This results in three samples collected per sampling day. Samples are preserved with ethanol in the field and shipped from the domain facility to a taxonomy lab for sorting and identification to lowest possible taxon (e.g., genus or species) and counts of each taxon per size are made to the nearest mm.

Data Wrangling Decisions The NEON zooplankton data product (DP1.20219.001) consists of dataframes for taxonomic identification and related field data. Zooplankton in NEON samples

are identified at contracting labs to the lowest possible taxonomic resolution, usually genus,
however some specimens can only be identified to the family (or even class) level, depending on
the condition of the specimen. Ten percent of all samples are checked by two taxonomists and
are noted in the qcTaxonomyStatus column. The taxonomic naming has been standardized in
the zoo\_taxonomyProcessed table, according to NEON's master taxonomy, removing any
synonyms. Density was calculated using adjCountPerBottle and towsTrapsVolume to correct
count data to "count per liter."

## Results (or how to get and use standardized NEON organismal data)

All cleaned and standardized datasets can be obtained from the R package neonDivData, which can be installed from Github (we will submit it to CRAN in the future). Installation instructions 718 can be found on the Github webpage (https://github.com/daijiang/neonDivData). Table 2 shows the brief summary of all data objects. To get data for a specific taxonomic group, we can just call the objects in the R object column in Table 2. Such data products include cleaned (and standardized if needed) occurrence data for the taxonomic groups covered and are equivalent to 722 the "observation" table of the ecocomDP data format. If environmental information and species measurements were provided by NEON for some taxonomic groups, they are also included in these data objects. Information such as latitude, longitude, and elevation for all taxonomic groups were saved in the neon\_location object of the R package, which is equivalent to the 726 "sampling\_location" table of the ecocomDP data format. Information about species scientific 727 names of all taxonomic groups were saved in the neon\_taxa object, which is equivalent to the 728 "taxon" table of the ecocomDP data format. To demonstrate the use of data packages, we used data\_plant to quickly visualize the 730 distribution of species richness of plants across all NEON sites (Fig. 2). To show how easy it is to 73 get site level species richness, we presented the code used to generate the data for Fig. 2 below.

Table 2: Summary of data products included in this study (as of 15 March, 2021). Users can call the R objects in the R object column from the R data package neonDivData to get the standardized data for specific taxonomic groups.

Taxa	R object	N species	N sites	Start date	End date
Algae	data_algae	1946	33	2014-07-02	2019-07-15
Beetles	data_herp_bycatch	758	47	2013-07-03	2020-10-13
Birds	data_bird	541	47	2015-05-13	2020-07-20
Fish	data_fish	147	28	2016-03-29	2020-12-03
Herptiles	data_herp_bycatch	128	41	2014-04-02	2020-09-29
Macroinvertebrates	data_macroinvertebrate	1330	34	2014-07-01	2020-07-29
Mosquitoes	data_mosquito	128	47	2014-04-09	2020-06-16
Plants	data_plant	6197	47	2013-06-24	2020-10-07
Small_mammals	data_small_mammal	145	46	2013-06-19	2020-11-20
Ticks	data_tick	18	41	2014-04-17	2019-03-18
Tick_pathogens	data_tick_pathogen	12	15	2014-04-17	2018-10-03
Zooplankton	data_zooplankton	157	7	2014-07-02	2020-07-22

Figure 2 shows the utility of the data package for exploring macroecological patterns. One of the

most well known and studied macroecological patterns is the latitudinal biodiversity gradient,
wherein sites are more speciose at lower latitudes relative to higher latitudes (Fischer 1960,
Hillebrand 2004). Herbaceous plants of NEON generally follow this pattern, but interestingly,
Read et al. (2018) found that NEON small mammal data do not support this pattern.

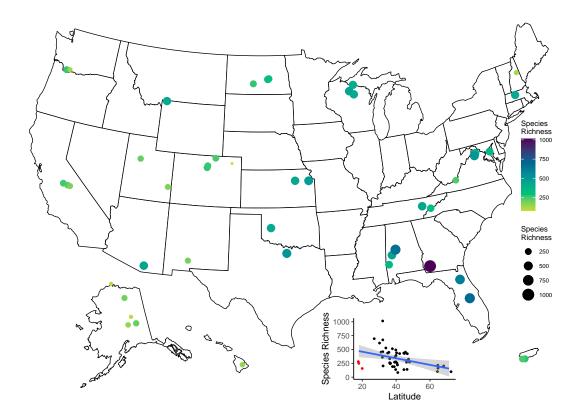


Figure 2: Plant species richness mapped across NEON terrestrial sites. The inset scatterplot shows latitude on the x-axis and species richness on the y-axis, with red points representing sites in Puerto Rico and Hawaii.

In addition to allowing for quick exploration of macroecological diversity patterns, the data
packages presented in this paper also enable investigation of effects of taxonomic resolution on
diversity indices since taxonomic information is preserved for observations under family level
for all groups. The degree of taxonomic resolution varies for NEON taxa depending on the
diversity of the group and the level of taxonomic expertise needed to identify an organism to the
species level, with more diverse groups presenting a greater challenge. Beetles are one of the
most diverse groups of organisms on Earth and wide-ranging geographically, making them ideal
bioindicators of environmental change (Rainio and Niemelä 2003). To illustrate how the use of

the beetle data package presented in this paper enables NEON data users to easily explore the effects of taxonomic resolution on community-level taxonomic diversity metrics, we calculated Jost diversity indices (Jost 2006) for beetles at the Oak Ridge National Laboratory (ORNL) NEON site for data subsetted at the genus, species, and subspecies level. To quantify biodiversity, we used Jost indices, which are essentially Hill Numbers that vary in how abundance is weighted 750 with a parameter q. Higher values of q give lower weights to low-abundance species, with q = 075 being equivalent to species richness and q = 1 representing the effective number of species given by the Shannon entropy. These indices are plotted as rarefaction curves, which assess the sampling efficacy. When rarefaction curves asymptote they suggest that additional sampling will not capture additional taxa. Statistical methods presented by Chao et al. (2014) provide estimates 755 of sampling efficacy beyond the observed data (i.e., extrapolated values shown by dashed lines in 756 Fig. 3). For the ORNL beetle data, Jost indices calculated with higher values of q (i.e., q > 0) 757 indicated sampling has reached an asymptote in terms of capturing diversity regardless of 758 taxonomic resolution (i.e., genus, species, subspecies). However, rarefaction curves for q = 0, which is equivalent to species richness do not asymptote, even with extrapolation. These plots suggest that if a researcher is interested in low abundance, rare species, then the NEON beetle 761 data stream at ORNL may need to mature with additional sample collections over time before 762 confident inferences may be made, especially below the taxonomic resolution of genus.

## Discussion (or how to maintain and update standardized NEON organismal data)

NEON organismal data hold enormous potential to understand biodiversity change across space
and time (Balch et al. 2019, Jones et al. 2021). Multiple biodiversity research and education
programs have used NEON data even before NEON became fully operational in May 2019 (e.g.,
Farrell and Carey 2018, Read et al. 2018). With the expected long-term investment to maintain
NEON over the next 30 years, NEON organismal data will be an invaluable tool for
understanding and tracking biodiversity change. NEON data are unique relative to data collected
by other similar networks (e.g., LTER, CZO) because observation collection protocols are

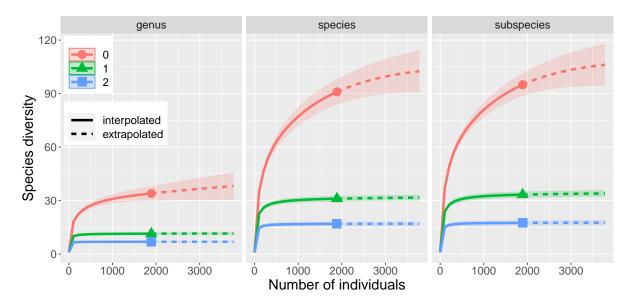


Figure 3: Rarefaction of beetle abundance data from collections made at the Oak Ridge National Laboratory (ORNL) National Ecological Observatory Network (NEON) site from 2014-2020 generated using the beetle data package presented in this paper and the iNEXT package in R (Hsieh et al. 2016) based on different levels of taxonomic resolution (i.e., genus, species, subspecies). Different colors indicate Jost Indices with differing values of q (Jost 2006).

standardized across sites, enabling researchers to address macroscale questions in environmental 773 science without having to synthesize disparate data sets that differ in collection methods (Jones 774 et al. 2021). The data package presented in this paper holds great potential in making NEON data 775 easier to use and more comparable across studies. Whereas the data collection protocols 776 implemented by NEON staff are standardized, the decisions NEON data users make in wrangling their data after downloading NEON's open data will not necessarily be similar unless the user 778 community adopts a community data standard, such as the ecocomDP data model. Adopting 779 such a data model early on in the life of the observatory will ensure that results of studies using 780 NEON data will be easier to compare and synthesize. By providing a standardized and 781 easy-to-use data package of NEON organismal data, our effort here will significantly lower the barriers to use the NEON organismal data for biodiversity research by many current and future 783 researchers and will ensure that studies using NEON organismal data are comparable. 784 There are some important notes about the data package we provided. First, our processes assume 785 that NEON already did its best to make sure that species identifications are correct. Since records may be identified to any level of taxonomic resolution, and IDs above the genus level may not be

relatively easier to identify (i.e., fish, plant, small mammals) or have very few taxon IDs that are above genus level (i.e., mosquito). However, for groups that are hard to identify (i.e., algae, beetle, bird, macroinvertebrate, tick, and tick pathogen), we decided to keep all records no matter what level of taxon IDs they have. Such information can be useful if we are interested in questions such as species-to-genus ratio or species rarefaction curves at different taxonomic levels (e.g., Fig. 793 3). Users thus need to think carefully about which level of taxon IDs they need for their research. Another note regarding species names is the term 'sp.' vs 'spp.' across NEON organismal data collections. The term 'sp.' refers to a single morphospecies whereas the term 'spp.' refers to more than one morphospecies. This is an important point to consider for community ecology 797 biodiversity analyses because it may add uncertainty into estimates of biodiversity metrics such 798 as species richness. Second, we kept records without any observed species/individuals in some taxonomic groups (i.e., beetle, bird, small mammal), thus preserving "true zeroes." Such empty 800 records provide critical information about sampling efforts. For example, if two sites both have the same number of small mammals during the same time period, however, one site has many more empty records (traps), then we can infer that the abundances of small mammals are lower 803 than the other site. If such information is not needed in a project, then they can be removed. Third, for algae, birds, and beetles, we added standardized measurements as density (algae) and number of fish caught per hour or number of beetles caught per trap night in the data package. And finally, there are other organismal groups (Aquatic Microbes and Aquatic Plants; soil microbes are covered in Qin et al. in this issue) not included in this study given the complexity of 808 the data. 800 All code for the Data Wrangling Decisions are available within the R package ecocomDP (Github and Zenodo, <a href="https://github.com/EDIorg/ecocomDP">https://github.com/EDIorg/ecocomDP</a>). Users can modify the code if they need to 811 make different decisions during the data wrangling process and correct any mistakes in our code 812 by submitting a pull request to our Github repository. If researchers wish to generate their own 813 derived organismal data sets from NEON data with slightly different decisions than the ones outlined in this paper, we recommend that they use the ecocomDP framework, contribute their workflow to the ecocomDP R package, upload the data to the EDI repository, and cite their data 816 with the discoverable DOI given to them by EDI. Note that the ecocomDP data model was

useful for most biodiversity projects, we removed records with such IDs for groups that are

intended for community ecology analyses and may not be well suited for population-level analyses. 819 Because ecocomDP is an R package to host code to harmonize different datasets following the ecocomDP format, we developed an R data package neonDivData to host and distribute the standardized NEON organismal data derived from ecocomDP. A separate dedicated data package 822 has several advantages. First, it is easier and readily to use and saves time for users to run the 823 code in ecocomDP to download and standardize NEON data products. Second, it is also easy to 824 update the data package when new raw data products are uploaded by NEON to their data portal; and the updating process does not require any change in the ecocomDP package. This is ideal 826 because ecocomDP harmonizes many different datasets besides NEON data. Third, the Github 827 repository page of neonDivData can serve as a discussion forum for researchers regarding the 828 NEON data products without competing for attention in the ecocomDP Github repository page. By opening issues on the Github repository, users can discuss and contribute to improve our workflow of standardizing NEON data products. Users can also discuss whether there are other 831 data models that the NEON user community should adopt at the inception of the observatory. As 832 the observatory moves forward, this is an important discussion for the NEON user community 833 and NEON technical working groups to promote synthesis of NEON data with data from other efforts (e.g., LTER, CZO, Ameriflux, the International LTER, National Phenology Network, Long 835 Term Agricultural Research Network). 836 The derived data products presented here collectively represent hundreds of hours of work by 837 members of our team - a group that met at the NEON Science Summit in 2019 in Boulder, 83 Colorado and consisting of researchers and NEON science staff. Just as it is helpful when 839 working with a dataset to either have collected the data or be in close correspondence with the 840 person who collected the data, final processing decisions were greatly informed by conversations with NEON science staff and the NEON user community. Future opportunities that encourage

collaborations between NEON science staff and the NEON user community will be essential to

achieve the full potential of the observatory data.

843

## 845 Conclusion

Macrosystems ecology (sensu Heffernan et al. 2014) is at the start of an exciting new chapter with the decades long awaited buildout of NEON completed and standardized data streams from all sites in the observatory becoming publicly available online. As the research community embarks on discovering new scientific insights from NEON data, it is important that we strive to make our analyses and all derived data as reproducible as possible to ensure that connections 850 across studies will be possible. Harmonized data sets will help in this endeavour because they 851 naturally promote the collection of provenance as data are collated into derived products 852 (O'Brien et al. In review, Reichman et al. 2011). Harmonized data also make synthesis easier 853 because efforts to clean and format data leading up to analyses do not have to be repeatedly performed by individual researchers (O' Brien et al. In review). The data standardizing processes 855 and derived data package presented here illustrate a potential path forward in achieving a 856 reproducible framework for data derived from NEON organismal data for ecological analyses. 857 This derived data package also highlights the value of collaboration between the NEON user community and NEON staff for advancing NEON-enabled science.

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