Environmental DNA Metabarcoding Effectively Detects Invasive Species, Pests, and Community Changes in Taiwan's Rice Fields

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Abstract

Rice fields represent man-made semi-aquatic wetlands primed for invasive pests. Monitoring rice field biodiversity using conventional methods is time-consuming and laborious. Environmental DNA (eDNA) methods can provide a fast and effective means to monitor rice field communities and inform management decisions. Our study provides proof-of-concept of rice field eDNA biodiversity assessments, with a focus on native and non-native pests across cultivation phases. We collected eDNA samples from locations in southern Taiwan during planting and harvesting, employing eDNA metabarcoding (COI) to detected diverse taxonomic groups. We assigned 78 ASVs across all sites to animal taxa, 34 of which were identified to species. Overall, 18 species were designated as native or non-native (83.3% and 16.6%, respectively), including three major rice pests, *Chilo suppressalis* (native), *Coptotermes formosanus* (native), and *Pomacea canaliculata* (non-native). Cultivation affected overall diversity, with higher species richness during planting compared to harvesting. No significant differences were observed between native and non-native taxa between cultivation phases. Altogether, we detected a complex environment across trophic levels comprised of both native and non-native agricultural pests using limited sampling effort, demonstrating eDNA as an efficient biomonitoring approach in rice agroecosystems with direct applications for pest, invasive species, and vector surveillance within Taiwan.

Keywords

Agriculture; invasive species; eDNA; molecular ecology; rice pest; biodiversity monitoring

Introduction

Rice fields represent man-made, wetland agroecosystems that support a wide range of biodiversity, from microorganisms to macroinvertebrates and amphibians (Bambaradeniya & Amarasinghe, 2004). Moreover, their alternating hydrologic dynamics, from flooded during the wet seasons to dry conditions in between, mean rice fields house a unique array of biodiversity (Watanabe et al., 2013). Additional ecological characteristics, such as nutrient-rich environments, human influences, and the effects of climate change, collectively make these ephemeral wetlands particularly vulnerable (Flanagan et al., 2015). Major threats to the ecological stability and biodiversity in the rice fields include anthropogenic pressures (e.g., intensive monoculture, agrochemical use) and the spread of invasive species (Tilman et al., 2002; Horgan, 2023).

In Taiwan, rice is one of the major staple crops, deeply embedded in the economy and culture, with almost half of the arable land consisting of rice fields (Hsing, 2014). However, rice fields are one of the most susceptible places for a myriad of invasive pests (Valls et al., 2014), and given the country's subtropical climate and extensive irrigation networks, several invasions of non-native species pose serious threats to both crop productivity and native biodiversity (Barker, 2002; Huang et al., 2010; Wu et al., 2010). Several invasive pest species severely impact rice cultivation in Taiwan; for example, the introduction and establishment of *Pomacea* spp. have become serious threats to rice agriculture (Barker, 2002; Wu et al., 2010), and are currently responsible for large economic losses throughout Asia (Jiang et al., 2022). Migratory rice planthoppers (*Nilaparvata lugens*) (Otuka et al., 2012) and the recently introduced fall armyworm (*Spodoptera frugiperda*) (Tsai et al., 2000) are also major economic concerns. Compounding impacts, native pests, such as rice stem borers (*Chilo suppressalis*, *Sesamia inferens*, and *Scirpophaga incertulas*), planthoppers (e.g., *Sogatella furcifera*) (Huang et al., 2010), leafhoppers (e.g., *Nephotettix* spp.), and the rice

67 black bug (*Scotinophara coarctata*), additionally contribute to considerable crop damage (Cheng
68 et al., 2010, 2022; Triapitsyn et al., 2021).

69 Conventional methods of biomonitoring, such as dipnet and funnel traps, are well
70 established and effective for understanding the co-occurrence of organisms associated with rice
71 cultivation (Song & Kuo, 2022), particularly invertebrates. However, conventional methods are
72 time-consuming and require taxonomic expertise, limiting the scale and scope needed for
73 establishing large-scale temporal monitoring needed to enable adaptive resource management
74 (Baird & Hajibabaei, 2012). Environmental DNA (eDNA)-based metabarcoding has recently
75 revolutionized biodiversity monitoring by enabling the non-destructive detection of targeted
76 and/or multiple species directly from environmental samples (Taberlet et al., 2012; Valentini et
77 al., 2016; Didaskalou et al., 2022). This relatively new approach has proven valuable in agricultural
78 ecosystems, where ecological interactions, including both beneficial and pest organisms, can be
79 monitored for proper management decisions (Kestel et al., 2022; Llanos et al., 2025). However,
80 its broad-scale implication in agricultural practices remains limited (Kestel et al., 2022) to a few
81 studies that have implemented eDNA methods to understand the general community associated
82 with rice fields (Ushio et al., 2023; Katayama et al., 2024; Vastano et al., 2024). Previous studies
83 have established species-specific approaches for an early detection method for cryptic invasive
84 gastropods (Banerjee et al., 2022) and fall armyworm (Tsai et al., 2020). However, more robust
85 and community-level monitoring is necessary for early management action that goes beyond
86 species-specific targets.

87 Recent advancements in high-throughput sequencing (HTS) technologies have enhanced
88 the resolution of eDNA metabarcoding approaches, enabling detailed taxonomic and functional
89 assessments of communities present within agricultural environments (Valentini et al., 2016;

90 Seymour et al., 2021; Ushio et al., 2023; Katayama et al., 2024; Vastano et al., 2024). Our study
91 aimed to elucidate a comprehensive understanding of co-occurring species associated with rice
92 field cultivation across two phases of rice cultivation (i.e., planting and harvesting) in Taiwan. To
93 do so, we implemented an eDNA-based metabarcoding approach targeting the mitochondrial
94 cytochrome c oxidase I (COI) gene to investigate the community dynamics of native, non-native,
95 and pest species associated with various rice fields. As environmental conditions (e.g., pH, salinity,
96 temperature, and other abiotic factors) putatively influence the shaping of community composition
97 and function within agroecosystems (Hartmann & Six, 2022) and potentially also modulate eDNA
98 detectability and persistence (Barnes & Turner, 2016; Stewart, 2019), we co-measured water and
99 soil parameters alongside our eDNA sampling.

100 We hypothesized that (i) environmental DNA (eDNA) would be an effective method for
101 detecting the vast array of interactive animal species from rice fields via water samples; and (ii)
102 during the planting season, species diversity would be high, but community composition would
103 demonstrate a higher proportion of colonizing non-natives and pests compared to harvesting time
104 due to the drier environment.

105

106 **Materials and Methods**

107 **Sample collection and environmental parameter analysis**

108 We collected eDNA samples from eight rice fields in Chiayi County, Taiwan (Figure 1, Table 1).
109 To understand the changes in the community during rice growth, including native and non-native
110 species, all fields were visited two times: (i) planting phase (denoted as samples "PF1-PF8") which
111 consist of the period of time at the beginning of the growing season where crops are seeded and
112 vegetative growth initiates, and (ii) harvesting phase ("HF1-HF8"), characterized by the ripening

113 phase, with mature rice seedling and dry environment. In our study, planting phase sampling
114 occurred on 22 February 2022, and the harvesting phase sampling occurred on 31 May 2022. We
115 collected five 500 mL surface water samples from each field (N = 40 samples per season). Water
116 samples were kept on ice and transported directly to the Department of Earth and Environmental
117 Sciences, National Chung Cheng University, for further analysis.

118 After collection of eDNA, water chemistry was recorded at each site including pH,
119 dissolved oxygen (DO) (mg/L), temperature (°C), salinity (PSU), oxidation-reduction potential
120 (ORP) (mV), conductivity (mS/cm), total dissolved solids (TDS) (mg/L), and resistivity (Ω-cm)
121 using a Hanna multiparameter waterproof meter (HANNA instruments, model HI98194, USA),
122 replicating each measurement ten times per site. Subsequently, the water sample was acidified
123 with 5 M HNO_3 to achieve pH < 2, followed by filtration using 0.22-µm nylon syringe filters
124 (13 mm; PureTech™, Taiwan) into 50-ml centrifuge tubes for further heavy metal analysis (see
125 the analytical methods below). Additionally, 3 x 300 g soil samples were collected from each site
126 to measure the physicochemical properties (such as pH and percentage of sand, silt, and clay with
127 texture) and heavy metal concentration, following protocols outlined in Dey et al. (2022). Soil pH
128 was measured using a 1:2.5 soil-to-deionized water suspension with a pH meter. Soil particle size
129 distribution was analyzed by the hydrometer method and classified into textural classes following
130 USDA-NRCS guidelines (Bouyoucos, 1962).

131 Concentrations of elements Bi, Cd, Mn, Cu, Ni, Co, Cr, Sr, Zn, Pb, and Fe in filtered acid-
132 digested water samples were analyzed using Inductively Coupled Plasma Optical Emission
133 Spectrometry (ICP-OES; Agilent Technologies 5100 ICP-OES, CA, USA). To ensure accuracy
134 and precision, a negative control ultrapure water (PP1-185UV Type I, UNISS, Taiwan) and a
135 positive reference standard were included (ICP multi-element standard solution IV). The recovery

136 rates for the analyzed metals ranged between 94% and 115%, with precision maintained under a
137 10% relative standard deviation (RSD) for all elements. The soil samples were dried at 70°C for
138 48 hours, followed by grinding in a mortar and pestle, and then were prepared for heavy metal
139 concentrations analysis using ICP-OES (Agilent Technologies 5100 ICP-OES, USA), based on
140 the method described by Bakshi et al. (2018). Briefly, 0.4 g dried soil was digested with 8 ml aqua
141 regia (a 3:1 mixture of HCl and HNO3) and 2 ml 40% hydrofluoric acid (HF). After cooling, the
142 solution was diluted with 30 mL of deionized water and then filtered with 0.22 µm nylon syringe
143 filters (13 mm; PureTech™, Taiwan) before the ICP-OES analysis.
144
145 **eDNA Filtration, Extraction, and Amplification**
146 In the laboratory, all water samples were passed through a GN-6 Metricel membrane mixed
147 cellulose ester filter paper (GN-6 Metricel ®, Pall Corporation, USA) with a diameter of 47 mm
148 and a pore size of 0.45 µm. As rice field water has high TDS, any clogging issues were mitigated
149 by using multiple filter papers as sub-samples (e.g., Hunter et al. 2019). Once our filtration was
150 complete, half of each filter paper was processed for immediate eDNA extraction, and the latter
151 half was preserved at -20 °C in Longmire's buffer for future use (Longmire et al., 1997). For
152 samples that required multiple sub-filters to mitigate clogging, all filter papers corresponding to
153 the same sample were placed in a single tube for DNA extraction. During the filtration process,
154 three filtration blanks (distilled water) were included to determine contamination and false positive
155 detection during laboratory analysis. Sterilization processes were maintained throughout the
156 workflow, like (i) frequent decontamination with 70% ethanol and 10% bleach, (ii) unidirectional
157 workflow with each step (i.e., filtration, DNA extraction, PCR, and post-PCR) performed in a
158 separate space, and (iii) PCRs were performed in a well-maintained hood.

159 A DNeasy Blood & Tissue kit (Qiagen, Germany) was used to extract eDNA from filters
160 according to the manufacturer's protocol, but with some modifications as follows: (i) the filter
161 papers were incubated at 60°C overnight in a mixture of 180 µL of ATL lysis buffer and 20 µL
162 Proteinase K; and (ii) the elution step was performed twice, where the spin columns were incubated
163 with the same 50 µL elution buffer at 37°C for 10 minutes (Spens et al., 2017). The quality and
164 quantity of extracted DNA samples were assessed using a Nanodrop spectrophotometer (Implen,
165 Germany) and an Invitrogen Qubit 3.0 fluorometer (Thermo Fisher Scientific, USA). For
166 budgetary reasons, a pooling approach was used for sequencing (Melcher et al., 2024). After
167 extractions, all subsamples (5 samples from each field) were pooled together based on their
168 quantity, thus making 8 pooled samples for each planting (n = 8) and harvesting (n = 8). Finally,
169 these 16 pooled samples were used for further downstream analysis.

170 A portion of mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified with
171 the following primers: mlCOIintF- 5′- GGWACWGGWTGAACWGTWTAYCCYCC- 3′ and
172 jgHCO2198 - 5′-TAIACYTCIGGRTGICCRAARAAYCA- 3′, specially designed for metazoans
173 (Leray et al., 2013). The reaction mixture of 25 µL for conventional PCR was prepared by mixing
174 5 µL 5x Fast-Run^{TM} Taq Master Mix with Dye (Protech Technology Enterprise, Taiwan), 0.5 µL
175 each 10 pmol forward and reverse primers (Genomics, Taiwan), 3 µL eDNA sample, and adding
176 high-quality sterile distilled water (Thermo Fisher Scientific, USA) to make up the final volume.
177 For PCR, we tried to add 3 µL eDNA instead of 1µL to allow the potential to amplify all pooled
178 samples. Three PCR replicates for each sample (including blank samples) were carried out. The
179 PCR reactions were performed with the following conditions: an initial denaturation at 94°C for 5
180 min, followed by a continuous amplification process of 36 cycles, with denaturation at 94°C for
181 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min. The final extension was at

182 72°C for 5 min after the completion of 36 cycles. The PCR products were visualized in a 1.5%
183 agarose gel electrophoresis, and all subsamples/replicates were mixed in equal density ratios
184 (based on band intensity) using the Gel Doc XR system with the QUANTITY ONE™ software
185 (Bio-Rad, USA). No visible amplification was observed in any blank sample (filtration or
186 extraction blanks); these were therefore excluded from further analysis. The PCR products were
187 then purified using 1.5× SPRI beads (AMPure XP; Beckman Coulter, USA). Purified amplicons
188 were quantified using an Invitrogen Qubit 3.0 fluorometer and submitted for library preparation
189 and sequencing at Genomics Bioscience and Technology Co., Ltd., Taiwan.
190

191 **Library preparation and next-generation sequencing**

192 The library was prepared using the TruSeq Nano DNA Library Prep Kit (Illumina, USA), and its
193 quality was assessed using an Agilent Bioanalyzer 2100 system (Agilent Technologies, USA). The
194 target inserts (~313 bp of the mitochondrial COI gene fragment) were derived from the PCR
195 amplification described earlier. The final library size, including Illumina adapters, was
196 approximately 450-500 bp, as measured by Bioanalyzer. Libraries were individually indexed
197 (barcoded) during preparation and pooled (multiplexed) in equimolar concentrations before
198 sequencing. Library quantification was performed using qPCR to ensure accurate equimolar
199 pooling. All qualified libraries were sequenced using 300 bp paired-end reads on an Illumina
200 MiSeq platform (Illumina, USA), operated by Genomics Bioscience and Technology Co., Ltd.,
201 Taiwan. Sequencing was carried out on a multiplexed standard v3 MiSeq flow cell.
202

203 **Data processing**

204 Raw sequencing data were demultiplexed using an in-house script developed by Genomics
205 Bioscience and Technology Co., Ltd., Taiwan, to assign reads to their corresponding samples
206 based on barcode sequences. Trimmomatic (v0.39) was used to remove the adapter sequences, and
207 low-quality bases (QV < 3) using with the following parameters:
208 ILLUMINACLIP:adapter:2:30:10:1:true LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15.
209 This configuration removes Illumina adapter sequences (allowing up to two seed mismatches),
210 trims low-quality bases from both ends of the reads, and applies sliding window trimming when
211 the average Phred quality score within the window falls below 15 (Bolger et al., 2014). Sequences
212 corresponding to both ends of the mlCOIintF and jgHCO2198 primers were trimmed using
213 Cutadapt (v3.4) (Martin, 2011). Primer trimming was performed with a minimum read length
214 cutoff of 150 bp to remove short, low-information reads, and reads lacking primer sequences were
215 discarded to ensure that only primer-containing sequences were retained. The trimming allowed a
216 minimum overlap of 3 bp and an error rate of 0.1 for primer matching. Denoising, chimera
217 removal, and assignment to Amplicon Sequence Variants (ASVs) were performed using DADA2
218 (Callahan et al., 2016), following the approach of (Sigsgaard et al., 2021). After error removal, all
219 remaining sequences were then compared against the GenBank nucleotide database (Sayers et al.,
220 2022) (as of September 2022) using BLASTN, and taxa were selected based on the Lowest
221 Common Ancestor (LCA) method (https://github.com/timkahlke/BASTA). The criteria for
222 sequence alignment included a maximum of 500 aligned sequences per query, with a ≥90% query
223 cover and 70-100% sequence similarity. For taxonomic assignments, we applied similarity
224 thresholds as follows: 80-84% for class-level, 85-89% for order-level, 90-94% for family-level,
225 95-97% for genus-level, and >98% for species-level (Sigsgaard et al., 2021). In cases where
226 multiple taxonomic matches occurred at a given level, the ASV was assigned to the next higher

227 taxonomic rank. The species detected via eDNA metabarcoding were classified as native or non-
228 native based on previous literature and the Catalog of Life in Taiwan (https://taicol.tw/).
229 Furthermore, the ecological roles were documented from previously published literature accessed
230 through Google Scholar, PubMed, and Web of Science.
231
232 **Statistical analysis**
233 All statistical analyses were performed using R version 4.3.3 (R Core Team, 2025). Alpha diversity
234 was estimated as observed ASV richness (number of unique ASVs per sample) and visualized
235 across field types using *ggplot2*. Differences in richness between cultivational phases were
236 assessed using Wilcoxon rank-sum tests. Beta diversity was assessed using non-metric
237 multidimensional scaling (NMDS) based on Bray–Curtis dissimilarities with the metaMDS()
238 function in the vegan package (Oksanen et al., 2001). Group differences in community
239 composition between cultivational stages were tested using PERMANOVA (*adonis2*). To
240 visualize the associations between species and cultivation phases (harvesting and planting), a
241 sankey diagram was constructed using the *networkD3* package (Allaire et al., 2014). All
242 visualization of taxonomic distributions and sample overlaps were performed using the *ggplot2*
243 and *eulerr* packages (Wickham, 2016; Yan, 2021). For the environmental parameter, mean values
244 ± standard error (SE) were calculated, and differences between harvesting and planting periods
245 were evaluated using Welch's two-sample t-test ($\alpha = 0.05$).
246
247 **Results**
248 **Community-level dynamics of rice fields**
249 **Sequencing reads and taxonomic composition**

250 We discarded one sample (HF2) due to low sequencing reads (< 3000). After filtering, 1,014,225
251 sequencing reads were retained, resulting in 67,615 ± 10,984 reads (mean ± standard error; n=15)
252 per sample. Overall, 196 amplicon sequence variants (ASVs) were initially identified across taxa,
253 including Metazoa, Protozoa, Algae, Fungi, and Bacteria. Of the total sequence reads, 54.92%
254 originated from Animalia (80 ASVs), 12.16% from Protozoa (25 ASVs), 4.24% from Bacteria (13
255 ASVs), 3.29% from Algae (38 ASVs), and 1.40% from Fungi (38 ASVs) (Table 2). Additionally,
256 23.95% of reads could not be taxonomically assigned with high confidence, as they fell below the
257 80% similarity threshold (2 ASVs) (Table 2). After removing the unassigned taxa (2 human ASVs
258 were also removed) and targeting animal taxa only (one ASV that could only identify up to
259 Animalia was removed), a total of 77 ASVs were retained for downstream analysis. The majority
260 of reads (93.12%) were from Arthropoda (43 ASVs), followed by Annelida (11 ASVs), Rotifera
261 (8 ASVs), Mollusca (5 ASVs), Chordata (4 ASVs), Gastrotricha (2 ASVs), Cnidaria (2 ASVs),
262 and Nematoda (2 ASVs) (Table 3). In total, 34 species were identified from various groups, and
263 among them, 18 species could be designated as either native or non-native based on published
264 literature, involving 83.3% native, 16.6% non-native (Table 4).
265
266 **Environmental parameters**

267 Soil pH was slightly alkaline (7.3–7.9) across sites, with textures ranging from sandy loam to
268 loamy sand. Particle size composition remained consistent across both cultivation times,
269 dominated by sand (approximately 72%). Heavy metal concentrations were found to exhibit
270 temporal variation, with Cd, Co, Cr, Cu, Ni, Zn, and Ag generally higher during harvesting, though
271 differences were not statistically significant. In contrast, both pH and Lead (Pb) were significantly
272 elevated in soil at harvest ($p < 0.05$; Table 5). Water parameters also reflected alkaline pH (7.52 ±

273 0.05 at harvesting, 7.54 ± 0.05 at planting) with relatively stable conductivity and salinity, though
274 dissolved oxygen increased notably during planting (5.26 ± 0.26 mg/L vs. 4.45 ± 0.02 mg/L at
275 harvesting). Temperature and TDS also showed significant differences, between cultivation phases
276 where temperature was significantly higher during the harvesting phase, and TDS was significantly
277 higher during the planting phase (Table 6). Trace metals in water showed differences in mean
278 values between stages; however, only the concentration of Lithium (Li) was statistically significant
279 (Table 6).

280 **Planting vs harvesting phase**

281 The relative abundance of the top 10 taxa at different taxonomic levels (order, family, genus, and
282 species) varied markedly between harvesting and planting phases (Figure 2). At the order level,
283 Diplostraca was the dominant order in both field types, accompanied primarily by Diptera in
284 harvesting fields and by Crassiclitellata and Cyclopoida in planting fields. At the family level,
285 Moinidae dominated both harvesting and planting fields, co-occurring with Culicidae and
286 Daphniidae in harvesting fields and with Megascolecidae and Cyclopidae in planting fields. At the
287 genus level, *Moina* sp., dominated both harvesting and planting fields, co-occurring with *Culex*
288 sp., and *Ceriodaphnia* sp., in harvesting fields and with *Metaphire* sp., and *Thermocyclops* sp., in
289 planting fields. At the species level, *Moina macrocopa* was dominant in both fields, accompanied
290 by *Culex tritaeniorhynchus* and Ceriodaphnia cornuta in harvesting fields and by *Metaphire*
291 *posthuma* in planting fields.

292 Of note, species overlap between planting and harvesting stages was substantial (44.1%;
293 Figure 3A) but also demonstrated some exclusive taxa between both cultivation phases, with more
294 unique species during the planting (35.7%) than the harvesting phase (14.3%) (Figures 3A, B).
295 This was corroborated by a Sankey diagram showing the association between the cultivation

296 phases and the relative abundance (based on sequencing reads) of taxa (Figure 3B). Moreover,
297 species richness was higher during the planting phase, with 12 native, 3 non-native, and 14
298 undetermined taxa, compared to 9 native, 2 non-native, and 9 undetermined taxa in the harvesting
299 phase (Figure 4). Still, the alpha diversity (based on ASVs) did not differ significantly between
300 harvesting and planting phases (p value = 0.52) (Figure 5A). The median species richness was
301 slightly higher during harvesting compared to planting phases, although the variation was larger
302 during planting, with a few high-diversity outliers (Figure 5A).

303 Despite no significant differences in alpha diversity across cultivation times, the non-
304 metric multidimensional scaling (NMDS) showed a pattern of differing community composition
305 between cultivation times (Figure 5B) (stress = 0.164). The PERMANOVA test confirmed that
306 the difference between the two growth phases was statistically significant ($p = 0.003$).
307 Additionally, no significant differences were observed between native and non-native taxa across
308 the two cultivation phases (Wilcoxon test, $p > 0.99$).

309

310 **Discussion**

311 In this study, we aimed to develop eDNA-based approaches for monitoring native and non-native
312 pest species in rice fields across different growth phases to aid in potential management strategies.
313 Overall, the eDNA-based method successfully detected a wide range of taxonomic groups during
314 both planting and harvesting phases, even with minimal sampling time and effort (Figure 3B, Table
315 4), consistent with the first hypothesis (H1). We successfully detected rice pests, including *Chilo*
316 *suppressalis* (native), *Coptotermes formosanus* (native), and *Pomacea canaliculata* (non-native),
317 among others (Table 4). Our results indicate that despite rice cultivation phases showing no
318 significant impact on species richness (Figure 5A), it does significantly impact community

319 composition (Figure 3A, B). We didn't observe any significant difference between native and non-
320 native taxa across the two cultivation phases (Figure 4), counter to our second hypothesis (H2).
321 However, this may also be a consequence of the relatively small sample size and limited study
322 area. Future studies incorporating a larger number of samples and broader spatial coverage would
323 improve the robustness of the analysis and may reveal patterns that were not detectable in the
324 present study.

325 Overall, our study demonstrated that a few liters of water from a rice field is sufficient to
326 amplify eDNA across interacting taxa, which could allow farmers or managers to implement quick
327 monitoring and response systems. In total, 77 ASVs were identified as animal taxa, and out of
328 them, 34 ASVs were assigned to species. Our limited number of identified species may also
329 suggest the necessity of building reference databases specifically for Taiwan for future eDNA-
330 based studies to increase accruacy and application (Schenekar et al., 2020).

331

332 **Functional role of the detected organism**

333 Our analysis of the rice field ecosystem revealed a diverse community spanning multiple trophic
334 levels, including primary consumers, detritivores, predators, omnivores, and native and non-native
335 agricultural pests (Table 4). Primary producers included rice crops and various phytoplankton.
336 Detritivores such as *Metaphire posthuma*, aquatic oligochaetes (*Allonais*, *Dero*, *Pristina*), soil
337 invertebrates (e.g., *Homidia socia*, *Coptotermes formosanus*), and gastropods (*Melanoides*
338 *tuberculata*) have an important role in nutrient cycling. Phytoplankton grazers included
339 cladocerans (e.g., *Moina macrocopa*, *Ceriodaphnia cornuta*), rotifers (*Brachionus*, *Keratella*), and
340 gastropods (*Pomacea canaliculata*, *Austropeplea ollula*). Omnivores such as ants (*Monomorium*
341 *pharaonis*, *Pheidole* sp.) and birds (*Passer montanus*, *Acridotheres tristis*) were also detected.

342 Secondary consumers included *Thermocyclops decipiens* and *T. taihokuensis*, while predators such
343 as frogs (*Microhyla fissipes*, *Fejervarya* sp.) and aquatic insects (*Diplonychus esakii*, *Microvelia*
344 *douglasi*) further structured the food web.
345 Two taxa of particular concern, *Chilo suppressalis* and *Pomacea canaliculata*, are major rice pests
346 responsible for economic damage (Chu, 1971); however, the detection of *Coptotermes formosanus*
347 is also a concern,as they may damage the rice plants (Li et al., 2009). We also recorded high
348 abundances of *Culex tritaeniorhynchus* (Figure 2), a vector of Japanese encephalitis virus, which
349 may raise concerns about the management of stagnant water in rice fields.
350
351 **Planting vs harvesting phase**
352 The NMDS results demonstrate that arthropod community structure differs significantly between
353 harvesting and planting phases. The tighter clustering of harvesting compared to the planting phase
354 suggests more homogeneous community assemblages under harvesting conditions, possibly due
355 to low availability of water or selective pressures associated with crop removal (Flannagan &
356 Marshall, 2012; Saksongmuang et al., 2024). In contrast, the broader spread of identified ASVs
357 during planting phases indicates greater community heterogeneity, which may reflect colonization
358 dynamics, variability in early vegetation structure, or differences in resource availability
359 (Mukherjee & Khan, 2017; Saksongmuang et al., 2024). This is also supported by environmental
360 parameters, where planting phases had higher DO, ORP, conductivity, and TDS compared to the
361 harvesting phase (Table 6).
362 The significant PERMANOVA results supports our proposition that these biodiversity
363 compositional differences are not random but instead driven by the phase of rice cultivation. This
364 may highlight that agricultural practices, even across short time scales, can influence community

365 composition, possibly through habitat modification, disturbance regimes, and resource distribution
366 (Mukherjee & Khan, 2017). The harvesting phases showed warmer conditions compared to the
367 planting phase, which aligns with the sampling time of the year and slight increases in soil pH and
368 Pb, probably due to cumulative agrochemical deposition.
369
370 **Native and non-native status**
371 Two frog species were identified as likely native to Taiwan. Among these, *Microhyla fissipes* is
372 recognized as native and widely distributed (Jang-Liaw & Chou, 2015). The other taxon,
373 *Fejervarya* sp., was identified only to the genus level, which includes both a native species
374 (*Fejervarya limnocharis*) and a non-native species (*Fejervarya cancrivora*) present in Taiwan
375 (Jang-Liaw & Chou, 2015; Lee et al., 2019). One native (*Passer montanus*) and an introduced bird
376 (*Acridotheres tristis*) with widespread distribution in Taiwan was also detected from the rice field
377 water (Ding et al., 2020). Among arthropods, *Coptotermes formosanus* is reported as a native pest
378 species (Li et al., 2009; Blumenfeld et al., 2021); however, this species is considered among the
379 top 100 invasive species in the world and its introduced population has been established in Japan,
380 Hawaii, and the southeastern United States (Simberloff & Rejmanek, 2019; Blumenfeld et al.,
381 2021). The native arthropod taxa detected included *Homidia socia*, *Culex tritaeniorhynchus*,
382 *Sciara humeralis*, *Diplonychus esakii*, *Microvelia douglasi*, *Monomorium pharaonis*, and *Chilo*
383 *suppressalis*. The non-native earthworm *Metaphire posthuma* was detected (Tsai et al., 2000); this
384 species is now naturalized and does not appear to negatively impact the local environment.
385 Additionally, one invasive snail (*Pomacea canaliculata*) which is responsible for various crop
386 damage, and two native snails (*Austropeplea ollula* and *Melanoides tuberculata*), were detected
387 together in the rice fields (Chang, 1991).

388 Although the sampling of rice field water provided a comprehensive assessment of the
389 species presence, the array of biodiversity detection was still less than other cprevious onventional
390 surveys conducted in Taiwan (Song & Kuo, 2022). This may be due to limitations in sampling
391 number, reference databases, and primer bias (Korbel et al., 2024). Thus, future studies should
392 implement a comparative analysis of conventional and eDNA methods to understand the potential
393 community shift during cultivation and the robustness of eDNA biomontioring compared to
394 conventional approaches.

395 Fewer detections with eDNA sampling compared to conventional approaches may also
396 suggest that the water sampling volume used here might not be sufficient to capture broad diversity
397 (Cantera et al., 2019). Frequent clogging of filter papers from eDNA in turbid habitats, such as
398 wetlands, is a key limitation (Hinlo et al., 2017). Future studies may look to test for pre-filtration,
399 pooling of extractions from multiple smaller volume samples, or implement passive filtration
400 methods (Stevens et al., 2024). Moreover, during the harvesting stage when less water is available,
401 eDNA approaches may also create bias due to the increased inhibitors within the water samples,
402 putatively impacting amplification and thus biodiversity recovery. Thus, additional soil and
403 swabbing, or washing of rice plants for eDNA, could possibly maximize detection. Still, our
404 eDNA-based approach detected a broad range of taxonomic groups with only limited sampling
405 effort and without requiring extensive taxonomic expertise. Thus, our method provides a fast and
406 effective tool for the regular monitoring of native and non-native pests in rice fields, and with
407 developing technology, it will be easy to use for the farmers. Furthermore, incorporating multiple
408 sample types (e.g., water, soil, leaf swabs), using group-specific primers, and increasing the
409 number of samples could further maximize the effectiveness of this monitoring approach.
410

411

412 **Conclusions**

413 Environmental DNA metabarcoding is an effective tool in revealing the diversity and composition
414 of communities in rice fields across Taiwan. Through this study, a wide range of taxa were detected
415 in a limited number of sampling events, providing valuable insights into the pest community. Three
416 detected pests (*Chilo suppressalis*, *Coptotermes formosanus*, and *Pomacea canaliculata*) are
417 highly concerning. Notably, the presence of non-native *Pomacea canaliculata* dominates over
418 native snails, raising concerns about the impact on native ecosystems and agricultural productivity.
419 Furthermore, the detection of disease vectors like the Japanese Encephalitis virus from the stagnant
420 rice field water is a rising concern for human health. These findings are particularly important for
421 sensitive agricultural systems where ecological and biological processes are becoming
422 increasingly recognized as essential for maintaining their ecological services. Overall, this study
423 highlights the transformative potential of eDNA metabarcoding for rapidly evaluating dynamic
424 biological and environmental systems.

425

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430 Sciences, National Chung Cheng University) for their help during the experiment.

431 **Authors' contributions**

P.B. conceived the idea; P.B., G.D., M.T., C.W.W., and R.K.S., performed experiments. P.B. prepared the first draft, and G.D., K.A.S., M.A.B., M.S., J.P.M., and C.Y.C. gave extensive edits and revised the manuscript, figures, and tables.

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Availability of data and materials

Sequence data will be available on request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Figure Captions

449 **Figure 1.** Location of the eight sampling sites near Chiayi County, Taiwan, where eDNA sampling
450 was conducted during February and May 2022.

451 **Figure 2**. Relative abundance of the top 10 taxa by field type. Stacked bar plots showing the
452 relative abundance (%) of the top 10 taxa detected in harvesting and planting field types, displayed
453 at four taxonomic levels: (A) orders, (B) families, (C) genera, and (D) species.

454 **Figure 3**. (A) Venn diagram of species detected in planting and harvesting fields. Circle and
455 overlap size denote proportional species diversity representation across field types. (B) Sankey
456 diagram showing the association between rice field types (planting and harvesting) and detected
457 species.

458 **Figure 4**. Functional roles and species status across rice field types. (Left) Relative abundance of
459 species categorized by functional roles during harvesting and planting. (Right) Species richness
460 grouped by published literature and the Catalog of Life in Taiwan (https://taicol.tw/) (Table 4;
461 native, non-native, and undermined/NA).

462 **Figure 5**. (A) Comparison of alpha diversity (observed ASVs) between field types (harvesting,
463 orange; planting, green) across sites (n=8). (B) Non-metric multidimensional scaling (NMDS) plot
464 based on Bray–Curtis dissimilarity showing beta diversity of communities between planting
465 (green) and harvesting (orange) rice fields detected from eDNA metabarcoding. Each point
466 represents a site, with ellipses indicating 95% confidence intervals for each field type.

467

468 **Table Caption**

Table 1. Metadata of rice field samples collected at two crop stages. Planting-stage samples (PF1–PF8) were collected on 10 February 2022, and harvesting-stage samples (HF1–HF8) were collected on 31 May 2022, from four rice fields located in Chiayi County, Taiwan. Coordinates for each sampling site are provided.

Table 2. Taxonomic distribution of amplicon sequence variants (ASVs) across major groups. The table summarizes the total number of reads, ASV richness, and percentage of reads assigned to each group. Unclassified reads with <80% similarity are denoted as "Below 80".

Table 3. Distribution of Animalia amplicon sequence variants (ASVs) across phyla. The table shows the total number of reads, ASV richness, and relative percentage of reads assigned to each phylum.

Table 4. Taxonomic composition, ecological roles, and native/non-native status of organisms detected in Taiwan rice field ecosystems. Species are categorized by phylum, with ecological roles spanning primary consumers, detritivores, predators, and omnivores; status and species type (native, non-native, or not available - N/A) assigned based on references.

Table 5. The physiochemical properties and heavy metal concentration in agricultural soil during harvesting and planting. p-value are from Welch's two-sample t-test (α=0.05). a and b subscripts represent significant differences.

Table 6. The physicochemical properties and heavy metal concentration of water at agricultural fields during harvesting and planting. p-value are from Welch's two-sample t-test (α=0.05). a and b subscripts represent significant differences.

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Table 1. Metadata of rice field samples collected at two crop stages. Planting-stage samples (PF1–PF8) were collected on 10 February 2022, and harvesting-stage samples (HF1–HF8) were collected on 31 May 2022, from four rice fields located in Chiayi County, Taiwan. Coordinates for each sampling site are provided.

Sample name	Field type	Location	Date
PF1	planting	23.567784, 120.455058	10-Feb-22
PF2		23.567432, 120.453490	
PF3		23.540607, 120.380341	
PF4		23.540305, 120.383952	
PF5		23.567776, 120.454035	
PF6		23.555544, 120.442193	
PF7		23.543891, 120.384877	
PF8		23.544579, 120.377238	
HF1	harvesting	Same as PF1	31-May-22
HF3		Same as PF3	
HF4		Same as PF4	
HF5		Same as PF5	
HF6		Same as PF6	

HF7	Same as PF7
HF8	Same as PF8

Table 2. Taxonomic distribution of amplicon sequence variants (ASVs) across major groups. The table summarizes the total number of sequencing reads, ASV richness, and percentage of reads assigned to each group. The majority of reads were assigned to Animalia, followed by unclassified reads with <80% similarity ("Below 80"), Protozoa, Bacteria, Algae, and Fungi.

Phylum	Total reads	ASVs	Percentage of reads
Animalia	557061	80	54.92
Below 80	242931	2	23.95
Protozoa	123431	25	12.16
Bacteria	43090	13	4.24
Algae	33448	38	3.29
Fungi	14264	38	1.40

Table 3. Distribution of Animalia amplicon sequence variants (ASVs) across phyla. The table shows the total number of sequencing reads, ASV richness, and relative percentage of reads assigned to each phylum. Arthropoda was the dominant phylum, followed by Annelida and Rotifera, while other phyla were represented at much lower abundances.

Phylum	Total reads	ASVs	Percentage of reads
Arthropoda	518717	43	93.12
Annelida	31958	11	5.73
Rotifera	3257	8	0.58
Mollusca	1614	5	0.28
Gastrotricha	779	2	0.13
Cnidaria	355	2	0.06
Nematoda	108	2	0.01
Chordata	53	4	~0.01

Table 4. Taxonomic composition, ecological roles, and native/non-native status of organisms detected in rice field ecosystems. Species are categorized by phylum, with ecological roles spanning primary consumers, detritivores, predators, and omnivores. Status is assigned based on literature, indicating whether species are native, non-native, or information not available (N/A).

Phylum	**Species**	**Ecological role**	**Status**
Annelida	*Metaphire posthuma*	Detritivores	Non-native (Tsai et al., 2000)
	Allonais sp.		N/A
	Bothrioneurum vejdovskyanum		N/A
	Dero sp.		N/A
	Pristina sp.		N/A
Arthropoda	*Ceriodaphnia cornuta*	Primary consumer	N/A
	Daphnia sp.	Primary consumer	N/A
	Moina macrocopa	Primary consumer	N/A
	Homidia socia	Detritivore	Native (Cheng et al., 2022)
	Thermocyclops decipiens	Secondary consumer	Native * (Reid, 1989)
	Thermocyclops taihokuensis	Secondary consumer	Native * (Reid, 1989)
	Coptotermes formosanus	Detritivores	Native (Blumenfeld et al., 2021)
	Tanypus kraatzi	Detritivores, and Omnivorous	NA
	Tanytarsus formosanus	Detritivores, and Omnivorous	Native (Gao et al., 2024)
	Culex tritaeniorhynchus	detritivore/filter-feeder (larva) and hematophagous (adult)	Native (Chung et al., 2024)
	Psilopa sp.	Detritivores	N/A
	Sciara humeralis	Detritivores (Larva), feed on nectar or fungi (adult)	Native
	Diplonychus esakii	Predator	Native (Rieger, 1995)

	Microvelia douglasi	Predator	Native (Ye et al., 2014)
	Monomorium pharaonis	Omnivorous	Native (Wetterer, 2010)
	Pheidole sp.	Omnivorous	N/A
	Chilo suppressalis	Primary consumer (Rice crop pest)	Native (Chu, 1971)
Chordata	*Microhyla fissipes*	Predator	Native (Jang-Liaw & Chou, 2015)
	Fejervarya sp.	Predator	N/A
	Passer montanus	Omnivorous	Native (Ding et al., 2020)
	Acridotheres tristis	Omnivorous	Non-native (Ding et al., 2020)
Gastrotricha	*Chaetonotus* sp.	Detritivores, microphagus	N/A
Mollusca	*Pomacea canaliculata*	Detritivorous herbivores. Primary consumer, Rice crop pest	Non-native (Banerjee et al., 2024)
	Austropeplea ollula	Detritivorous herbivores. Primary consumer	Native (Chang, 1991)
	Melanoides tuberculata	Detritivorous herbivores. Primary consumer	Native (Chang, 1991)
Rotifera	*Brachionus* sp.	Detritivores	N/A
	Keratella sp.		
	Lecane sp.		
	Polyarthra sp.		
	Rotaria sp.		

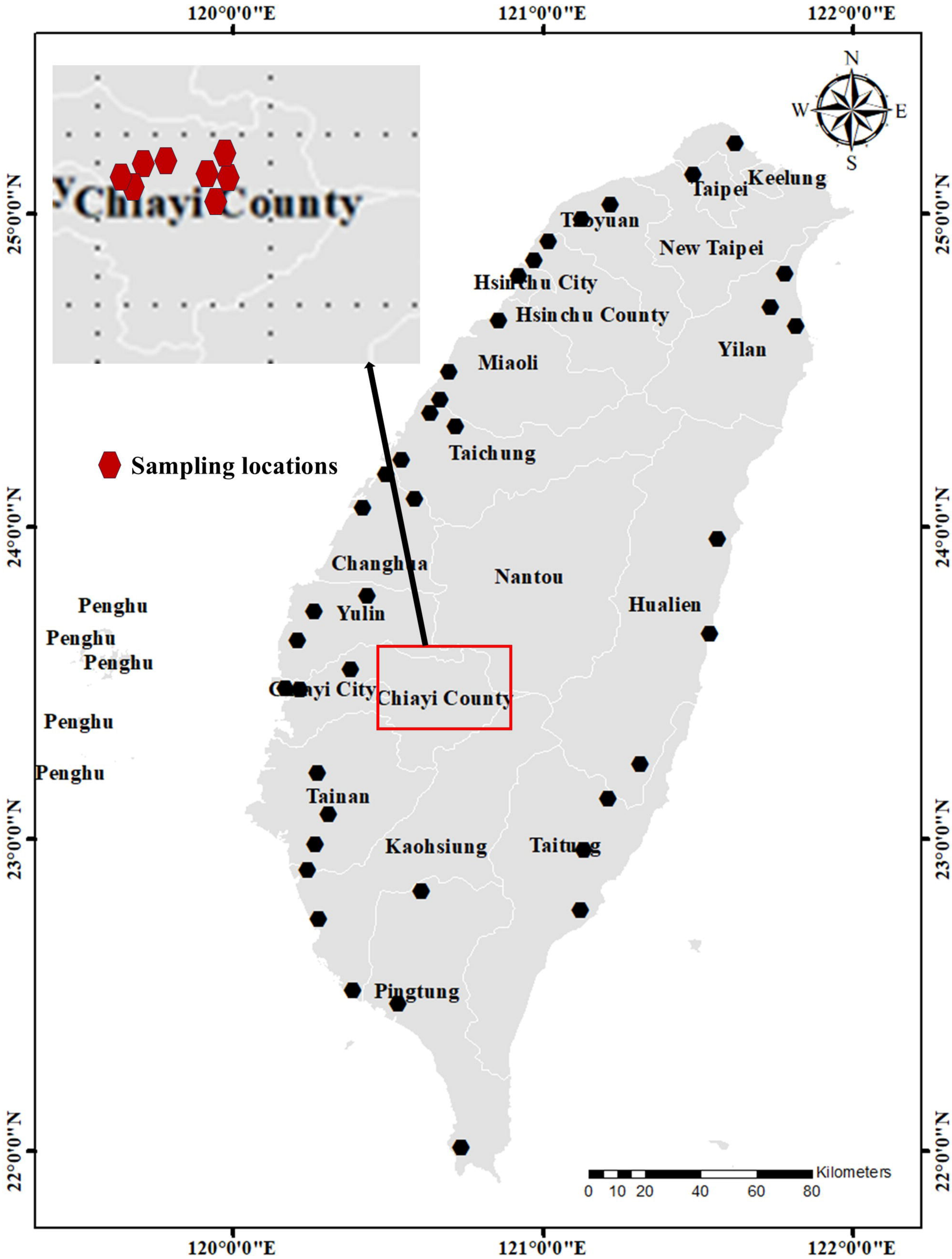
Table 5. The physiochemical properties and heavy metal concentration in agricultural soil during harvesting and planting. p-value are from Welch's two-sample t-test (α=0.05). a and b subscripts represent significant differences.

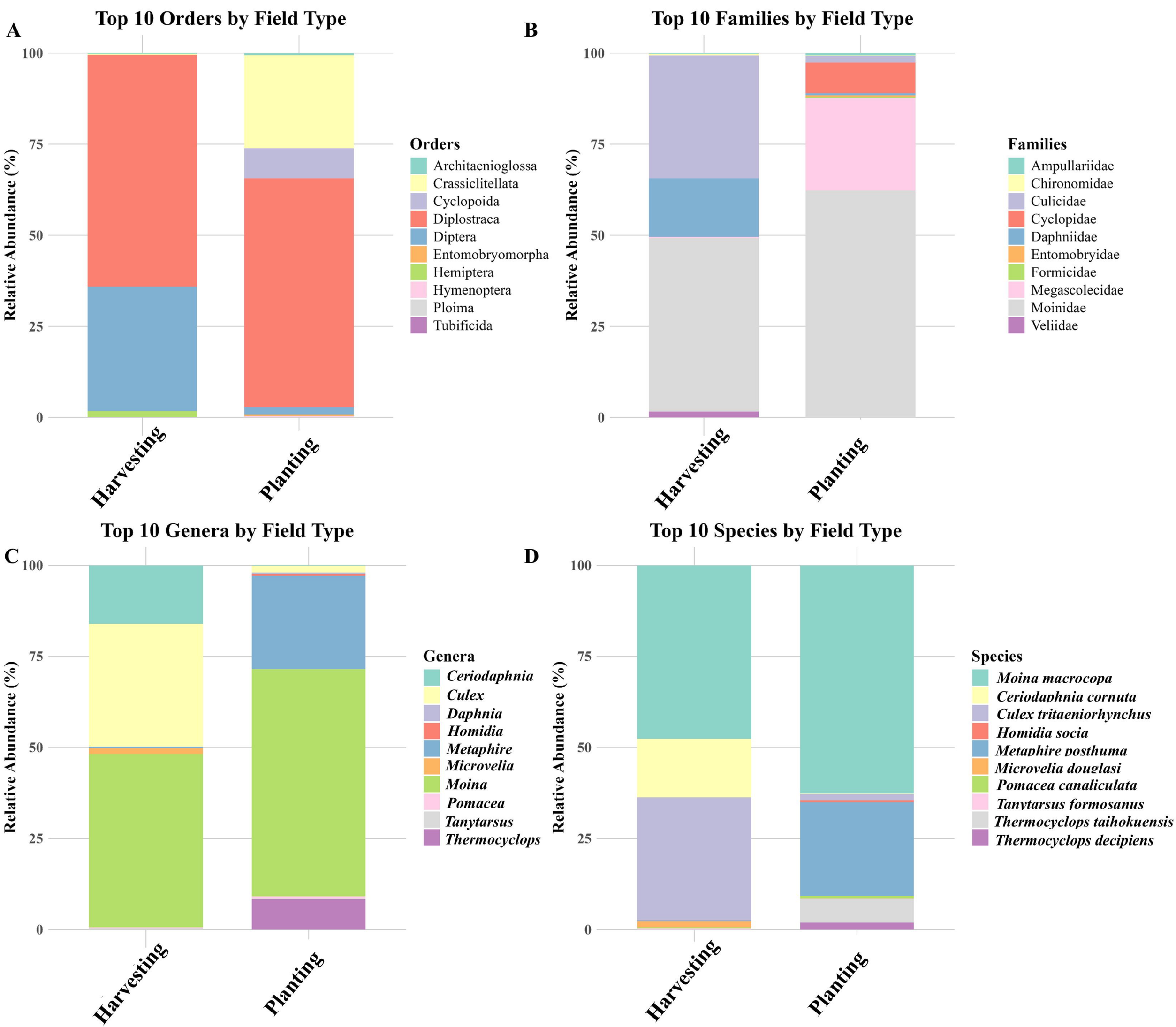
Parameter	Harvesting (Mean ± SE)	Planting (Mean ± SE)	p (Welch t-test)
pH	7.74 ± 0.05 [a]	7.54 ± 0.07 [b]	**0.0394**
Silt (%)	14.53 ± 1.69 [a]	15.15 ± 1.59 [a]	0.8075

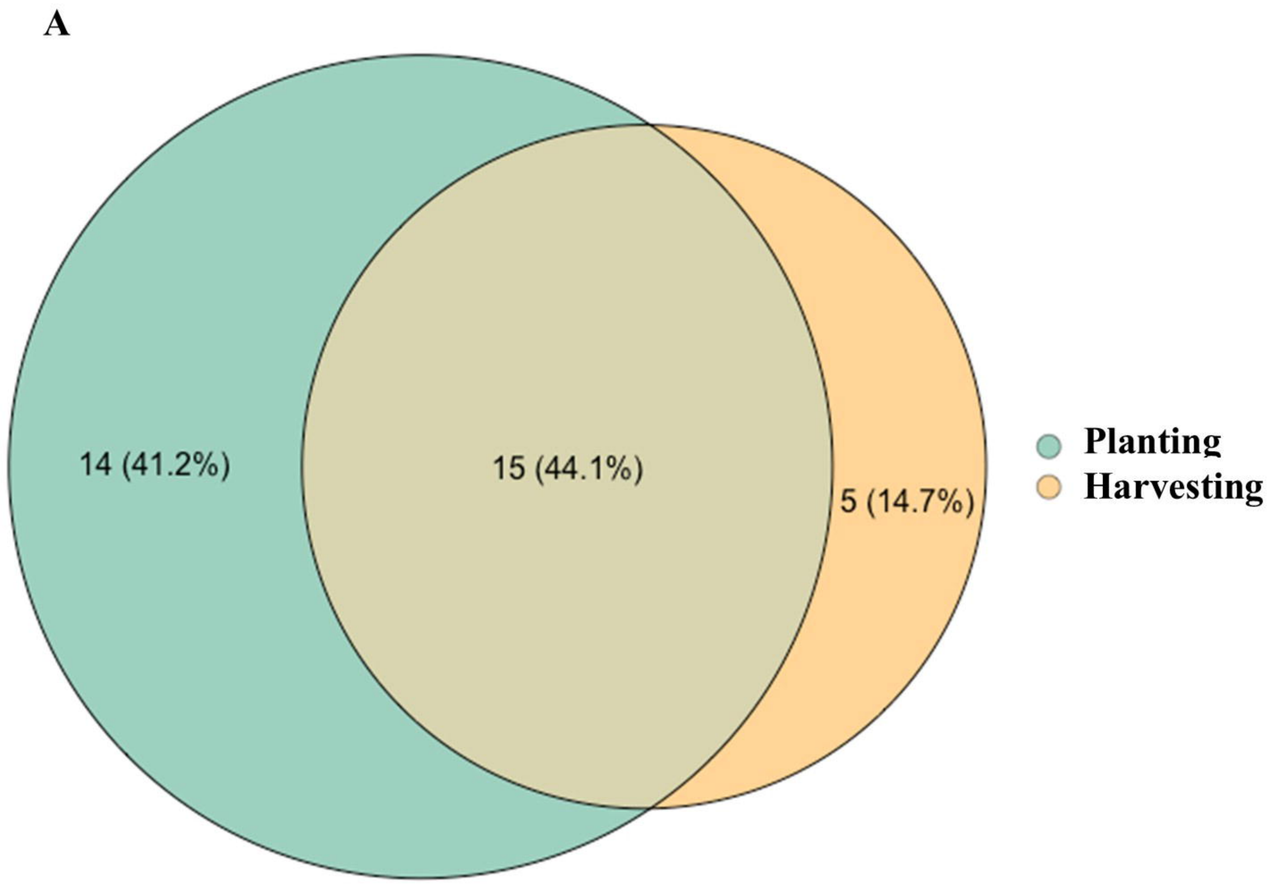
Clay (%)	13.10 ± 1.65 [a]	12.80 ± 1.47 [a]	0.9016
Sand (%)	72.37 ± 1.63 [a]	72.05 ± 1.46 [a]	0.8937
Cd (mg/kg)	0.44 ± 0.07 [a]	0.30 ± 0.06 [a]	0.1694
Co (mg/kg)	2.41 ± 0.57 [a]	2.02 ± 0.35 [a]	0.6029
Cr (mg/kg)	70.94 ± 6.48 [a]	64.66 ± 7.12 [a]	0.5536
Cu (mg/kg)	19.40 ± 1.77 [a]	16.46 ± 2.10 [a]	0.3379
Fe (mg/kg)	29781.25 ± 2729.60 [a]	24943.06 ± 2848.57 [a]	0.2743
Li (mg/kg)	37.34 ± 2.49 [a]	37.73 ± 1.77 [a]	0.9074
Mn (mg/kg)	256.67 ± 32.80 [a]	243.13 ± 36.91 [a]	0.8026
Ni (mg/kg)	35.11 ± 2.85 [a]	32.98 ± 3.93 [a]	0.6899
Pb (mg/kg)	35.81 ± 1.39 [a]	29.04 ± 2.49 [b]	**0.0492**
Sr (mg/kg)	269.93 ± 34.18 [a]	306.87 ± 27.48[a]	0.4489
Zn (mg/kg)	92.91 ± 9.04 [a]	86.12 ± 7.95[a]	0.6092
Ag (mg/kg)	1.26 ± 0.15 [a]	1.14 ± 0.21[a]	0.6702

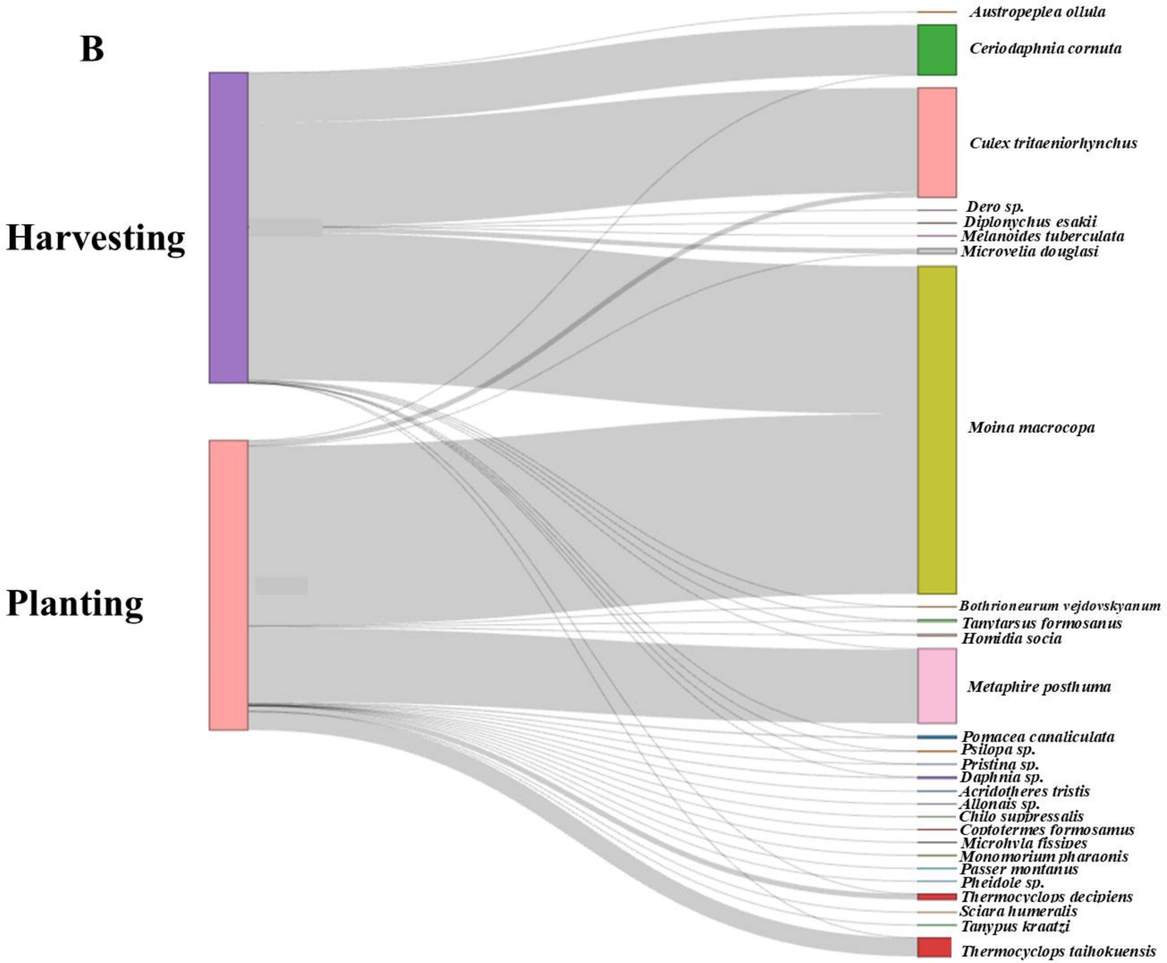
Table 6. The physicochemical properties and heavy metal concentration of water at agricultural fields during harvesting and planting. p-value are from Welch's two-sample t-test (α=0.05). a and b subscripts represent significant differences.

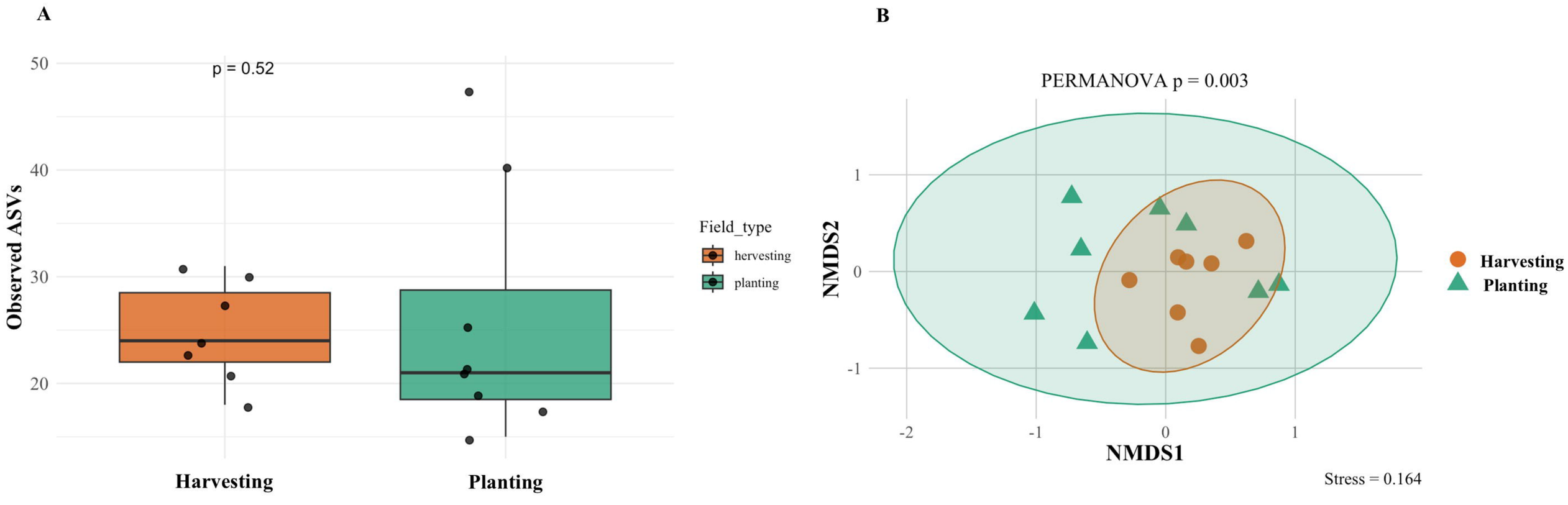
Parameter	**Harvesting (Mean ± SE)**	**Planting (Mean ± SE)**	**p (Welch t-test)**
pH	7.52 ± 0.06 [a]	7.54 ± 0.05 [a]	0.7818
mV pH	-21.48 ± 2.63 [a]	-26.70 ± 2.52 [a]	0.2059
ORP	152.37 ± 11.67 [b]	188.99 ± 3.77 [a]	**0.0270**
DO (mg/L)	4.45 ± 0.02 [b]	5.27 ± 0.26 [a]	**0.0197**
Conductivity (mS/cm)	0.40 ± 0.04 [b]	0.55 ± 0.05 [a]	**0.0363**
Resistivity (ohm·cm)	3070.52 ± 382.50 [a]	2111.05 ± 220.36 [a]	0.0723
TDS (mg/L)	187.08 ± 20.39 [b]	259.80 ± 22.12 [a]	**0.0424**
PSU	0.26 ± 0.02 [a]	0.25 ± 0.02 [a]	0.7992
Temperature (°C)	29.32 ± 0.88 [a]	26.08 ± 0.76 [b]	**0.0231**
Atm. Pressure (PSI)	14.57 ± 0.10 [a]	14.76 ± 0.02 [a]	0.1412
Fe (mg/L)	0.15 ± 0.07 [a]	0.03 ± 0.01 [a]	0.1676
Li (mg/L)	0.27 ± 0.00 [a]	0.26 ± 0.00 [b]	**0.0082**
Mn (mg/L)	0.31 ± 0.10 [a]	0.24 ± 0.11 [a]	0.6609
Sr (mg/L)	3.90 ± 0.87 [a]	6.61 ± 1.09 [a]	0.0938

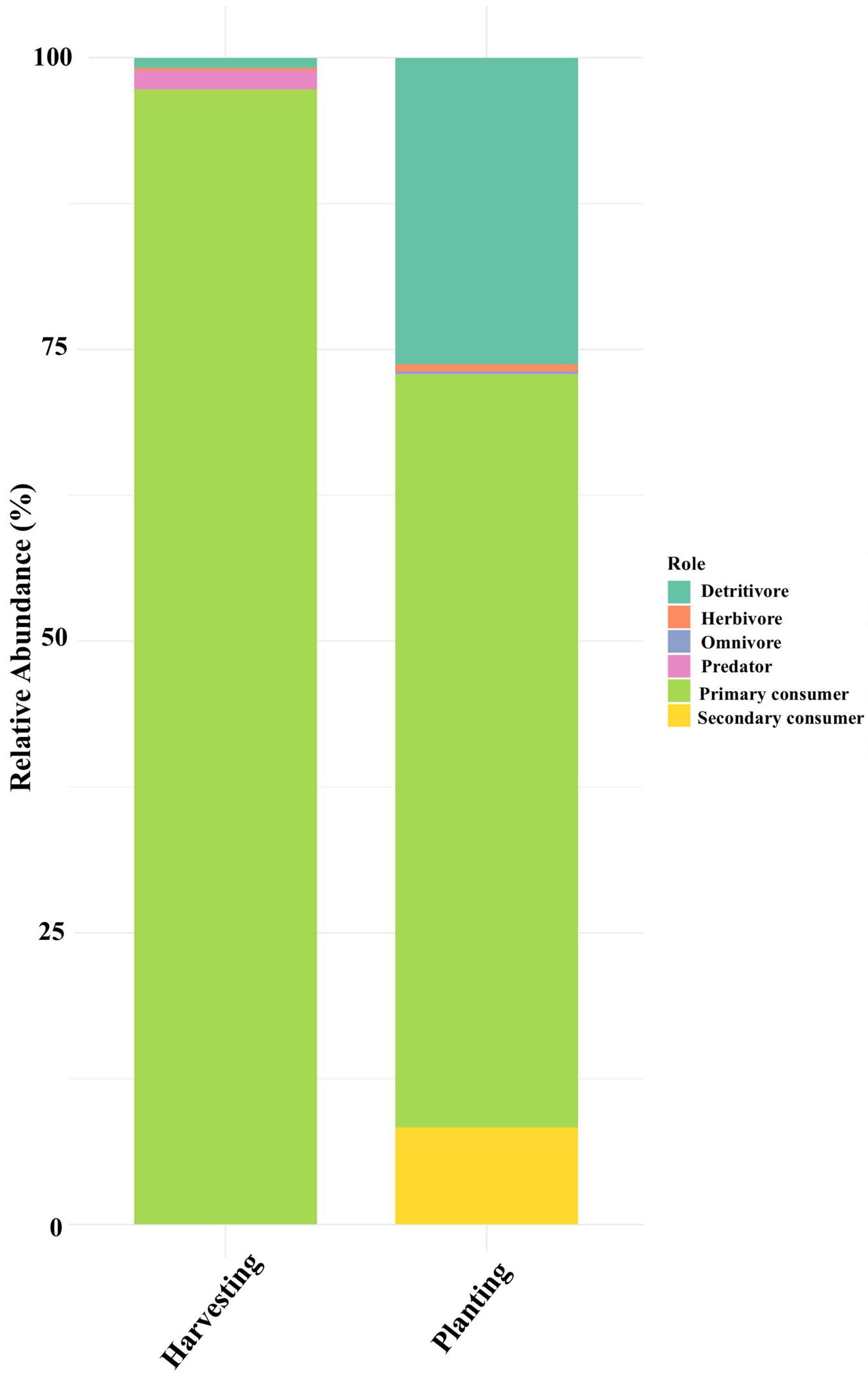

120°0'0"E
121°0'0"E
122°0'0"E
25°0'0"N
24°0'0"N
23°0'0"N
22°0'0"N
N
S
W
E
Chiayi County
Sampling locations
Keelung
Taipei
Taoyuan
New Taipei
Hsinchu City
Hsinchu County
Yilan
Miaoli
Taichung
Changhua
Nantou
Hualien
Yulin
Penghu
Chiayi City
Chiayi County
Tainan
Kaohsiung
Taitung
Pingtung
Kilometers
0 10 20 40 60 80

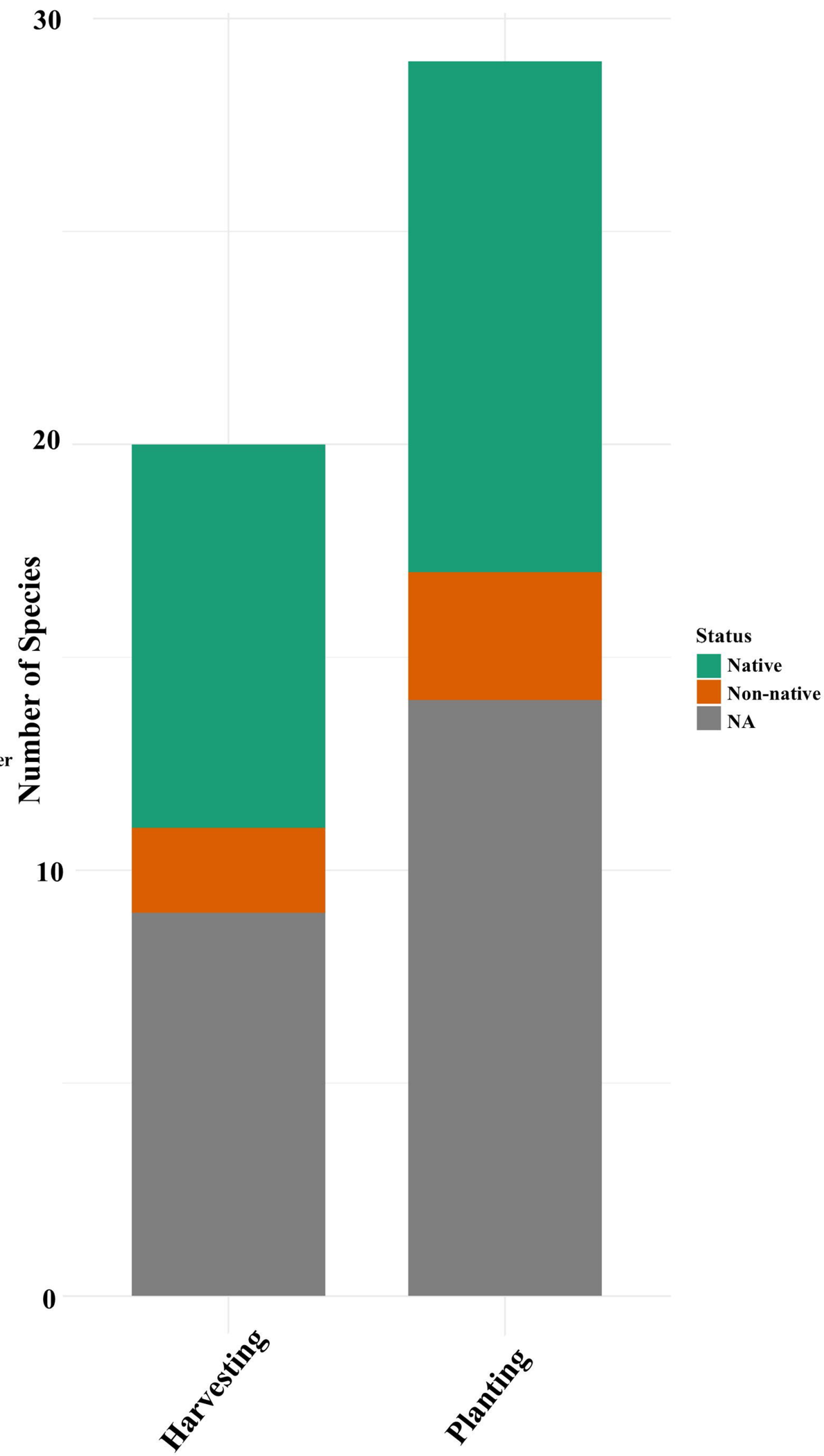

A
Top 10 Orders by Field Type
Relative Abundance (%)
100
75
50
25
0
Harvesting
Planting
Orders
Architaenioglossa
Crassiclitellata
Cyclopoida
Diplostraca
Diptera
Entomobryomorpha
Hemiptera
Hymenoptera
Ploima
Tubificida
B
Top 10 Families by Field Type
Relative Abundance (%)
Families
Ampullariidae
Chironomidae
Culicidae
Cyclopidae
Daphniidae
Entomobryidae
Formicidae
Megascolecidae
Moinidae
Veliidae
C
Top 10 Genera by Field Type
Relative Abundance (%)
Genera
Ceriodaphnia
Culex
Daphnia
Homidia
Metaphire
Microvelia
Moina
Pomacea
Tanytarsus
Thermocyclops
D
Top 10 Species by Field Type
Relative Abundance (%)
Species
Moina macrocopa
Ceriodaphnia cornuta
Culex tritaeniorhynchus
Homidia socia
Metaphire posthuma
Microvelia douglasi
Pomacea canaliculata
Tanytarsus formosanus
Thermocyclops taihokuensis
Thermocyclops decipiens


A
14 (41.2%)
15 (44.1%)
5 (14.7%)
Planting
Harvesting


B
Harvesting
Planting
Austropeplea ollula
Ceriodaphnia cornuta
Culex tritaeniorhynchus
Dero sp.
Diplonychus esakii
Melanoides tuberculata
Microvelia douglasi
Moina macrocopa
Bothrioneurum vejdovskyanum
Tanytarsus formosanus
Homidia socia
Metaphire posthuma
Pomacea canaliculata
Psilopa sp.
Pristina sp.
Daphnia sp.
Acridotheres tristis
Allonais sp.
Chilo suppressalis
Coptotermes formosanus
Microhvia fissipes
Monomorium pharaonis
Passer montanus
Pheidole sp.
Thermocyclops decipiens
Sciara humeralis
Tanypus kraatzi
Thermocyclops taihokuensis


A
p = 0.52
Observed ASVs
50
40
30
20
Harvesting
Planting
Field_type
hervesting
planting
B
PERMANOVA p = 0.003
NMDS2
NMDS1
1
0
-1
-2
-1
0
1
Harvesting
Planting
Stress = 0.164


100
75
50
25
0
Relative Abundance (%)
Harvesting
Planting
Role
Detritivore
Herbivore
Omnivore
Predator
Primary consumer
Secondary consumer


30
20
10
0
Number of Species
Harvesting
Planting
Status
Native
Non-native
NA