# Higher fungal diversity is correlated with lower $CO_2$ emissions from dead wood in a natural forest: BioRxiv preprint

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## Abstract

Wood decomposition releases almost as much $CO_2$ to the atmosphere as does fossil-fuel combustion, so the factors regulating wood decomposition can affect global carbon cycling. We used metabarcoding to estimate the fungal species diversities of naturally colonized decomposing wood in subtropical China and, for the first time, compared them to concurrent measures of $CO_2$ emissions. Wood hosting more diverse fungal communities emitted less $CO_2$, with Shannon diversity explaining 26 to 44% of emissions variation. Community analysis supports a 'pure diversity' effect of fungi on decomposition rates and thus suggests that interference competition is an underlying mechanism. Our findings extend the results of published experiments using low-diversity, laboratory-inoculated wood to a high-diversity, natural system. We hypothesize that high levels of saprotrophic fungal biodiversity could be providing globally important ecosystem services by maintaining dead-wood habitats and by slowing the atmospheric contribution of $CO_2$ from the world's stock of decomposing wood. However, large-scale surveys and controlled experimental tests in natural settings will be needed to test this hypothesis.


## Introduction

Global decomposition of wood releases $CO_2$ (6 to 9.5 Pg C/year[1, 2, 3]) at similar rates to fossil-fuel combustion (9.5 Pg C/year in 2011[4]). Decomposing wood also serves as essential habitat[5, 6]. The factors controlling wood decomposition rates are therefore of broad importance to conservation and to carbon cycle-climate feedbacks.

However, temperature and moisture variables only explain minority portions of total variance in decomposition rates[7, 8]. For instance, Bradford *et al*.[9] reported that regional temperatures explain only 28% of local variance in mass loss.

The diversity of wood-decomposing fungi might explain much of the remaining unexplained variance. In laboratory-inoculation experiments using small numbers of culturable fungal species, wood pieces with higher final fungal diversity exhibited reduced decay rates[10, 11, 12]. Inoculated wood placed in the field also showed a negative effect of final fungal species diversity on decay ($R^2 = 0.15$[13]).

However, in contrast to laboratory experiments, natural wood decomposition involves much higher species diversity, more complex assembly histories, and selective faunal feeding on decomposers[14, 15, 16]. Thus, it is important to examine the relationship between fungal diversity and decomposition rates in wood that is colonized and decomposing under natural conditions.

Natural fungal communities can be characterized using metabarcoding[17], in which nuclear ribosomal internal transcribed spacer (ITS) regions are PCR-amplified and read using high-throughput sequencing[18, 19, 20, 21]. ITS1 and ITS2 are each sufficiently variable to differentiate fungal species[18, 19] and return similar estimates of OTU (Operational Taxonomic Units) richness and community structure[19, 22].

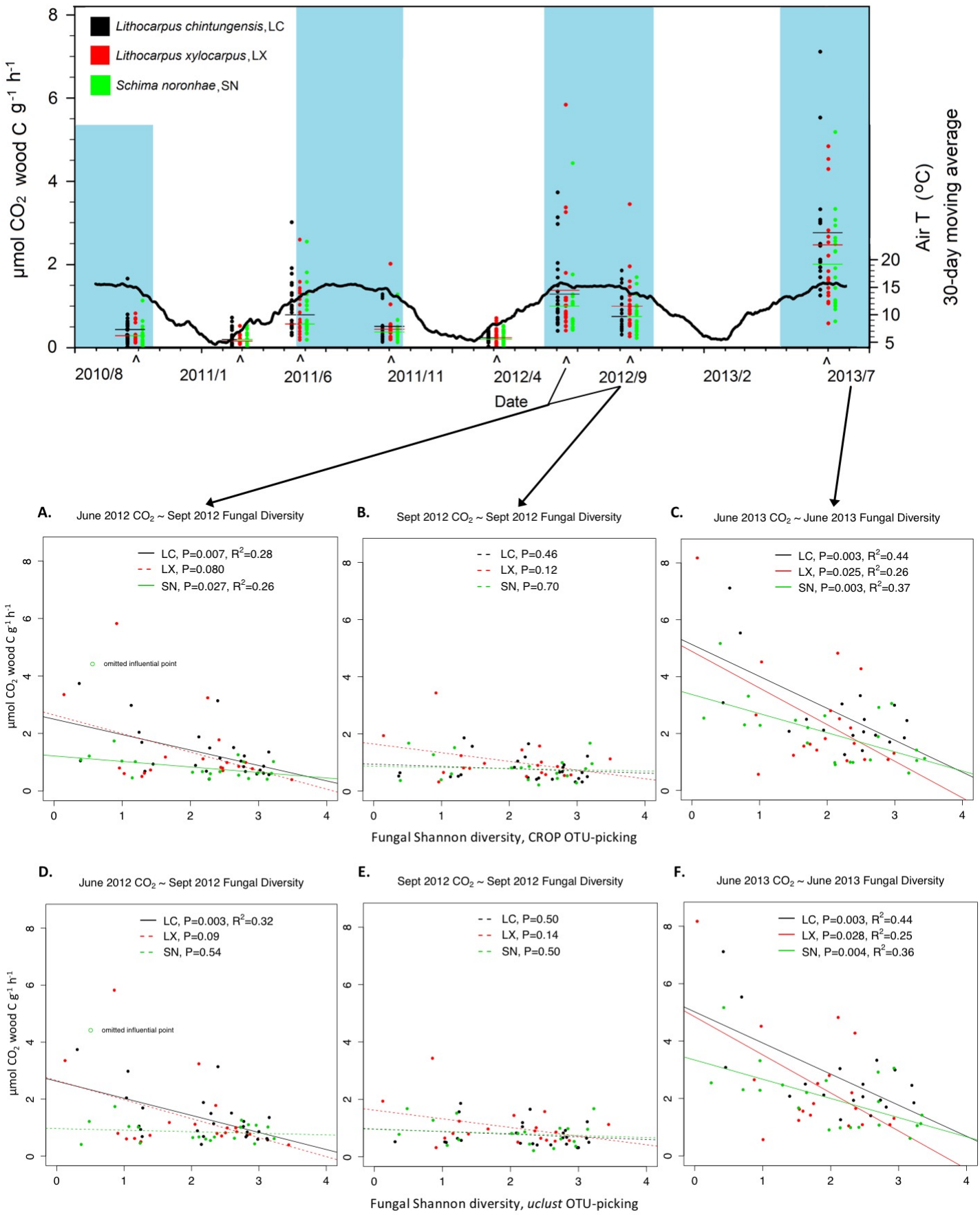
Here we metabarcoded ITS2 to examine fungal communities in naturally colonized wood pieces sampled across a wide range of decay classes in the Ailao Mountain forest of Yunnan, China. These wood pieces were sampled from a larger experiment involving three tree species (LC: *Lithocarpus chintungensis* [Fagaceae], LX: *L. xylocarpus*, and SN: *Schima noronhae* [Theaceae]) from which naturally occurring dead-wood pieces were regularly measured for $CO_2$ emission rates over three years[8]. We measured the extent to which variation in the species diversity and composition of fungal communities can explain variation in emission rates.

## Results

*Taxonomy results* – Numbers of fungal OTUs ranged from 17 to 199 across wood pieces, tree species, and sampling dates, with means of 73.8 (LC, Sep 2012), 76.7 (LC, June 2013), 87.0 (LX, Sep 2012), 90.5 (LX, June 2013), 83.7 (SN, Sep 2012), and 88.4 (SN, June 2013).

41.1% of the 1,807 OTUs produced by *uclust* and 76.3% of the 1,565 OTUs produced by CROP were assigned to Fungi, and the proportions assigned to each fungal class were similar across assignment methods (Table 1). Because we removed non-Fungi reads from the dataset before taxonomic assignment, we attribute the taxonomically unassigned OTUs to the still highly incomplete UNITE and Genbank databases used for taxonomic assignment.

*Fungal diversity and $CO_2$ emissions* – In June 2013, the month with the highest $CO_2$


Lithocarpus chintungensis, LC
Lithocarpus xylocarpus, LX
Schima noronhae, SN
µmol CO2 wood C g-1 h-1
Air T (°C)
30-day moving average
2010/8
2011/1
2011/6
2011/11
2012/4
2012/9
2013/2
2013/7
Date
A. June 2012 CO2 ~ Sept 2012 Fungal Diversity
LC, P=0.007, R2=0.28
LX, P=0.080
SN, P=0.027, R2=0.26
omitted influential point
B. Sept 2012 CO2 ~ Sept 2012 Fungal Diversity
LC, P=0.46
LX, P=0.12
SN, P=0.70
C. June 2013 CO2 ~ June 2013 Fungal Diversity
LC, P=0.003, R2=0.44
LX, P=0.025, R2=0.26
SN, P=0.003, R2=0.37
Fungal Shannon diversity, CROP OTU-picking
D. June 2012 CO2 ~ Sept 2012 Fungal Diversity
LC, P=0.003, R2=0.32
LX, P=0.09
SN, P=0.54
omitted influential point
E. Sept 2012 CO2 ~ Sept 2012 Fungal Diversity
LC, P=0.50
LX, P=0.14
SN, P=0.50
F. June 2013 CO2 ~ June 2013 Fungal Diversity
LC, P=0.003, R2=0.44
LX, P=0.028, R2=0.25
SN, P=0.004, R2=0.36
Fungal Shannon diversity, uclust OTU-picking

emissions, emissions declined with fungal diversity in all wood species ($R^2$ = 26% to 44%, Fig. 1C, F).

In June 2012, $CO_2$ emissions from LC and SN also declined with fungal diversity, even though we used a fungal diversity estimate taken three months later (September 2012) and even after conservatively omitting an influential datum from SN (high $CO_2$, low diversity) (Fig. 1A, D). The third species (LX) did not return a significant regression, but its $CO_2$-diversity relationship was visually nearly indistinguishable from its congener LC, suggesting that wood species partly governs the emissions-diversity relationship. Variances explained (26% to 28%, Fig. 1A, D) were lower than in June 2013.

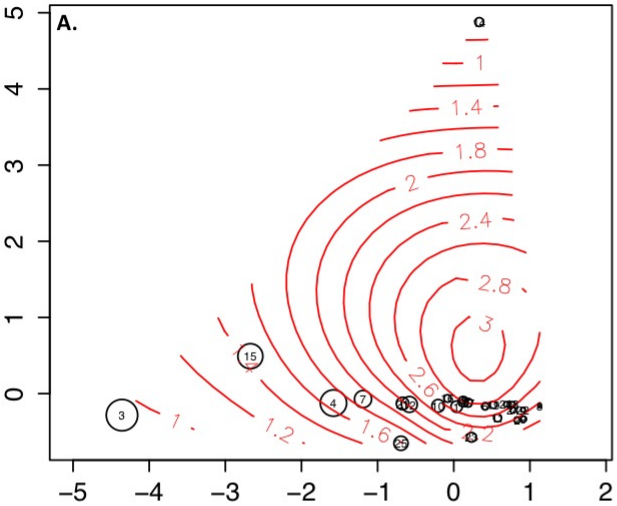
Finally, in September 2012, $CO_2$ emissions did not decline with higher fungal diversity (Fig. 1B, E), which is consistent with the generally lower $CO_2$ emissions in September (Fig. 1).

The above results were robust to two OTU-picking methods (CROP and *uclust*, Fig. 1), rarefaction (non-rarefied shown in Fig. 1; rarefied in Supporting Information S1), and two diversity estimates (Shannon in Fig. 1, Simpson in S1). Regressions using Simpson diversities were generally statistically *more* significant (S1). We also analyzed after omitting single-read OTUs (which are more likely to be pipeline artefacts[23]) and achieved the same results, except that the previously non-significant SN regressions in Sept 2012 (Fig. 1A, D) became statistically *significant* (authors' unpublished results). In short, the analyses presented in Fig. 1 are conservative.

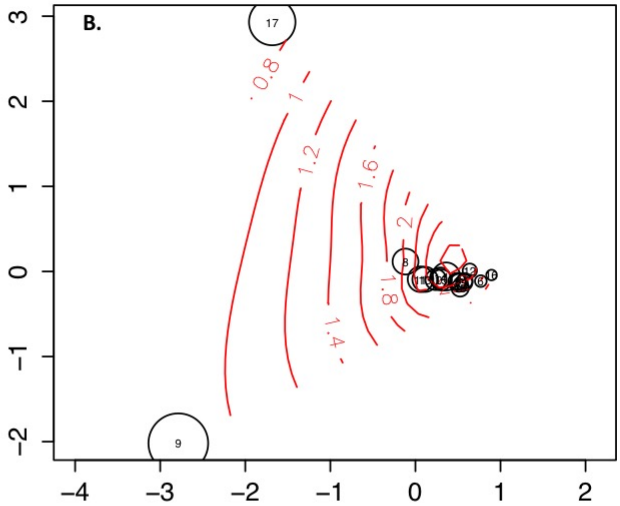
*Chemistry of decomposing wood* - We analyzed the chemistry of a separate subset of 27 wood pieces from the larger experiment. Mean $CO_2$ emissions from 2010-2012 showed no correlation with the densities of carbon, nitrogen, phosphorus, or lignin within any of the decay classes, with one exception, nitrogen density in decay class 1 (statistical details in Supporting Information S2).

*Pure-diversity versus species-selection effects* – Two general mechanisms could explain the observed diversity-function relationships. The first is a 'pure diversity' effect where species identity does not matter, only that increased species richness and evenness *per se* is somehow responsible for slower wood decomposition. The second is a 'species-selection' effect where more diverse fungal communities might be more likely to contain particular

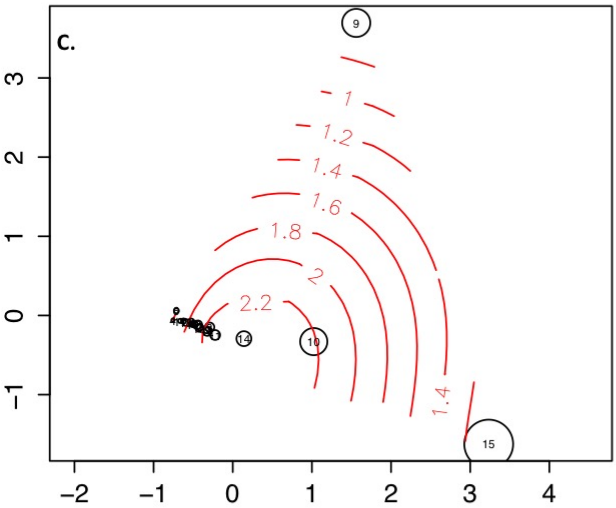
LC, June 2012 $CO_2$ ~ Sep 2012 Shannon



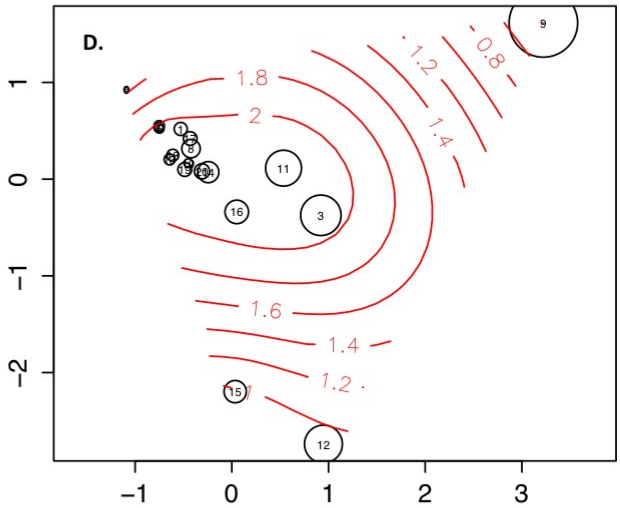

LC, June 2013 $CO_2$ ~ June 2013 Shannon



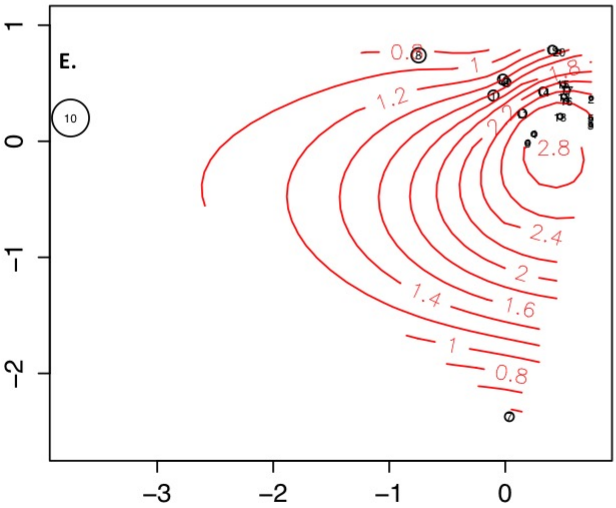

LX, June 2012 $CO_2$ ~ Sept 2012 Shannon



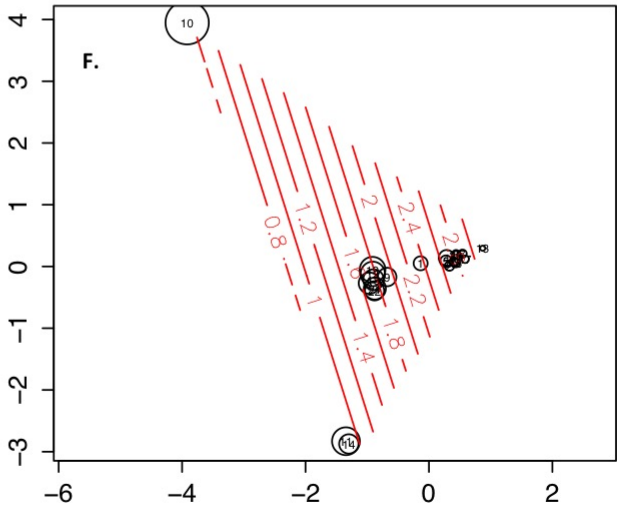

LX, June 2013 $CO_2$ ~ June 2013 Shannon




SN, June 2012 $CO_2$ ~ Sept 2012 Shannon




SN, June 2013 $CO_2$ ~ June 2013 Shannon



species that cause slow decomposition and somehow also govern the overall decomposition rate of the wood piece. To differentiate these two, we used a method devised by Sandau *et al*. [24] to generate a parameter λ for each regression in Fig. 1 (statistical details in Supporting Information S3). λ ranges between 0 and 1, with 0 indicating that variation in species composition does not account for variation in $CO_2$ emissions (i.e. a 'pure-diversity' effect). For two tree species, LC and LX, λ always took values near zero (Table 2). For the third tree species SN, λ was also nearly zero in June 2012 but took intermediate values in June 2013, suggesting that fungal composition in this tree species at this time had some explanatory power. The general failure to detect composition effects can be observed in the community ordinations (Fig. 2) by noting that the SN/June 2013 samples were the only ones to line up along the $CO_2$ emissions gradient (except the two lowest diversity samples). Not surprisingly, conventional community-analysis tests returned the same conclusion: variation in community composition is not explained by $CO_2$ emissions (statistical details in Supporting Information S4).

## Discussion

We found that naturally colonised wood with more diverse fungal communities decomposes more slowly (Fig. 1), resulting in a negative relationship between fungal biodiversity and the ecosystem function of decomposition. This result suggests positive relationships between fungal biodiversity and the ecosystem services of carbon storage and the provision of decomposing-wood habitat in forests.

Our results are consistent with five published experiments using laboratory-inoculated wood, which have all found negative relationships between fungal diversity and decomposition rates[10, 11, 12, 13, 25]. The one exception, Valentin *et al.*[26], found a positive relationship, but in that study, field-collected microbial communities were serially diluted and re-inoculated into laboratory wood incubations. Serial dilution does not necessarily remove microbial species, but it does make all species less abundant[27], which might have reduced decomposition rates.

In contrast, field studies to date have reported only ambiguous relationships between

fungal diversity and decomposition rates. For instance, Hoppe *et al*.[28] found non-significant correlations between fungal OTU richness and decay class (reflecting different numbers of years of decomposition), albeit negative relationships consistent with our results. Van Der Wal *et al*.[29] measured tree-stump decomposition and reported weakly *positive* effects of fungal species richness (but not Simpson diversity) on sapwood decomposition, but only in late decay. Kurbartová *et al*.[30] found no relationship between wood loss and fungal OTU diversity after 12 years of decomposition but also reported that the least-decayed logs had the highest community diversities, again consistent with our results. All three studies found differences in community composition for logs that differed in remaining undecomposed weights.

Importantly, none of those three field studies made concurrent measurements of fungal diversities and $CO_2$ emission rates, as we did here (Fig. 1). We observe that relationships between $CO_2$ emissions rate and fungal diversity varied from month to month and across tree species (Fig. 1, Table 2, S1), suggesting that fungal activity and composition are dynamic and environmentally responsive. Thus, the fungal community measured after years of decomposition might not reflect the communities that were active during decomposition, obscuring any relationship between mass loss and fungal diversity.

Our study helps to reconcile the differing results found in the published laboratory and field studies, by making concurrent measurements of emissions and fungal diversity in a field setting that is the most natural on the spectrum of possibilities: colonization of locally dominant tree species by the local fungal community, with uninterrupted and full exposure to local environmental variability and the local faunal community, including fungivores, and long-term succession of fungal and other microbial communities. Our collected wood pieces span a range of at least one to fifteen years of decomposition on the forest floor[8]. Our results suggest that laboratory experiments correctly reveal negative relationships between $CO_2$ emissions and fungal diversity.

*Mechanisms*. - In contrast to wood, microbial diversity is reported to accelerate the decomposition of soil organic matter[31,32], and this is thought to represent a general pattern[33,34] (but see Creed *et al.*[35] for leaf litter). We hypothesise that because soil organic matter presents a much higher diversity of resources than does dead wood, niche complementarity amongst

170 decomposer species drives positive relationships between diversity and soil organic matter
171 decomposition.

172 By contrast, niche overlap provides a plausible biological mechanism for why wood
173 decomposition should *slow* with fungal diversity. Interference competition has long been
174 predicted to evolve when niche overlap is high and the disputed resource is valuable[36]. In
175 forests, decomposing wood resources are available to many fungal species, and aggressive
176 interactions are indeed observed among these fungi[37]. Elsewhere, it has been shown that
177 interference competition reduces virulence (= host consumption rate) in endosymbioses[38.39,40]
178 and productivity in bacterial communities[41,42]. Consistent with those findings in other
179 contexts, interference competition can also explain why fungal biomass has been found to
180 explain variance in wood mass loss[9]. When a piece of wood is colonized by many fungal
181 species, the hypothesized higher levels of interference competition would result in less wood
182 converted into fungal biomass (or $CO_2$). High niche overlap is also consistent with the
183 observed 'pure diversity' effect of fungal diversity on emissions (Table 2, S3, S4) since any
184 species should fight all others (note, however, that we could not test for community
185 composition effects at higher taxonomic levels, see Maherali & Klironomos[43]). Thus, theory
186 suggests that the arrow of causation can be drawn in the direction of fungal diversity driving
187 decomposition rate. Finally, interference competition results in competitive exclusion, which
188 will cause community composition to change over time. This means that measures of fungal
189 diversity made after years of decomposition are unlikely to explain final variance in
190 decomposition.

191 *Sources of error and proposed future experiments*. - One source of error is that
192 metabarcoding provides only approximate estimates of species frequencies, due to the many
193 errors known to be introduced by metabarcoding, especially PCR-primer mismatches that
194 lead to biased amplification[22, 23]. Also, DNA is environmentally persistent, so fungal species
195 that are no longer represented by living colonies might still be detected by PCR. Nonetheless,
196 we found that Shannon and Simpson indices, which both incorporate species frequency
197 information by discounting rare species (here, rare=low-read OTUs), were able to explain
198 variation in $CO_2$ emissions. There are two likely and non-exclusive explanations. (1)
199 Low-read OTUs were more likely to have been the remains of dead species and/or sequence

artifacts from the metabarcoding pipeline and thus indeed should be discounted, and (2) in a Norway spruce forest, Ovaskainen *et al*.[21] found that abundances of fungal fruiting bodies and OTU read numbers were positively correlated, suggesting that low-read OTUs indeed represent low-biomass species, which should have weaker influence on decomposition rates.

Another possible source of error is that we did not experimentally control for the age of the wood pieces, and thus an alternative explanation is that the observed correlations between fungal diversity and $CO_2$ emissions rates (Fig. 1) might be caused by sampling along a successional gradient in which older wood pieces have less remaining wood to decompose (and thus lower emissions) and have also accumulated more fungal species. However, we found no relationship between decay class and emissions rates (*Methods: Experimental setup* and *Statistical analyses*) in our dataset, nor did we in the 320-piece superset from which our samples were drawn[8], whereas this alternative explanation predicts that the least-decayed wood pieces should show the highest emissions. Also, we found mostly 'pure-diversity' effects of fungal communities on emissions (Table 2, S3, S4), whereas this alternative explanation invokes a successional sere and thus predicts compositional effects. We suggest a long-term experiment in which even-aged and sterilized wood pieces are allowed to be colonized and sampled for $CO_2$ emissions rates and fungal diversity over many years in the field. We suggest that bacterial communities also be measured for correlations with $CO_2$ emissions, although we caution that to estimate alpha diversity, sequencing effort will need to be much higher than for fungal communities. In addition to DNA-based metabarcoding, it might also be informative to sequence reverse-transcribed RNA from wood samples, in order to isolate the effect of living fungal species.

*Conclusions*. - The slopes of our diversity-emissions relationships (Fig. 1) are steep enough to suggest that even modest declines in fungal diversity in dead wood could cause several-fold increases in $CO_2$ emissions rates. For example, in June 2013, $CO_2$ emissions varied by 5.6- and 14.4-fold among LC and LX wood pieces. These negative relationships between diversity and wood decomposition provide a strong justification to conduct large-scale surveys of the status of fungal biodiversity and its trajectories in the world's forests. Global forest fragmentation, reduction of tree-species diversity by fires, logging and replanting, the removal of dead trees, and even increased rainfall could all reduce fungal

230 biodiversity in forests[6,44]. All these changes could lead to faster wood decomposition. On the
231 other hand, fragmented forests are drier, fungal distributions are being globalized[45], and their
232 local diversity is increased by rising $CO_2$[46]. The net effect of all these changes on carbon
233 emissions from the world's stock of decomposing wood is difficult to predict.

## 234 Methods

235 *Site description.* – This study was conducted in the Ailao Mountains National Nature
236 Reserve, Yunnan, China, which preserves the largest area of undisturbed, subtropical moist
237 forest in China and has a substantial pool of woody debris (branches and logs, 74.9 x $10^3$ kg
238 $ha^{-1}$, Ref. 47). The study site was at an elevation of 2476 m, about 2 km north of the Ailao
239 Field Station for Forest Ecosystem Studies (24.533 ºN, 101.017 ºE), and receives 1840 mm
240 annual average precipitation. The climate is monsoonal with distinct cool/dry (November to
241 April) and warm/wet (May to October) seasons[48]. Annual mean air temperature is 11.3 ºC
242 with monthly means ranging from 5 to 16 ºC. Surface soils (0 to 10 cm) of the area are
243 Alfisols with pH of 4.2 (in water). The surficial organic layer is 3 to 7 cm deep[49]. The study
244 site is a broad-leaved evergreen subtropical forest, with the canopy dominated by *Lithocarpus*
245 *chintungensis, Rhododendron leptothrium, Vaccinium ducluoxii, Lithocarpus xylocarpus,*
246 *Castanopsis wattii, Schima noronhae, Hartia sinensis*, and *Manglietia insignsis*[50].
247 *Experimental setup.* – At our site, most woody debris comes from *Lithocarpus*
248 *chintungensis*, (LC), *Lithocarpus xylocarpus* (LX), and *Schima noronhae* (SN), so we only
249 examined those three species. In early 2010, branches from these three species, already
250 decomposing on the forest floor, were identified to species by a botanist from the
251 Xishuangbanna Tropical Botanical Garden, collected, and cut into a total of 320 wood pieces,
252 sized to fit a field-respiration chamber (*ca*. 10 cm diameter and 20 to 30 cm length), tagged,
253 weighed, and measured for size and decay class (further details in Liu *et al.*[8]). The three
254 decay classes were DKC1 = a knife could not penetrate, DKC2 = a knife could slightly
255 penetrate with appreciable resistance, DKC3 = a knife could deeply penetrate with little
256 resistance[51]. We used similar-sized pieces to control for potential effects of wood size on
257 fungal communities[52]. The pieces were placed on the forest floor within a 60 x 3 m belt

transect following an elevation contour. We collected source wood from within 500 m of this transect, utilizing about 5% of downed woody debris from these species in these decay classes, potentially arising from about 6000 source trees (D.A. Schaefer, unpublished data).

Each wood piece was initially weighed with a GLL portable electronic balance (accuracy 0.5 g) and for moisture content with an Extech MO210 moisture meter (calibrated as in Liu *et al.*[8]). Their volumes were calculated as cylinders, based on length and the average of 5 circumferential measurements along their lengths. From those, initial weight, volume, and density were all calculated. Oven drying of these wood pieces was not done, because it would have altered microbial communities and wood chemistry.

*$CO_2$ emissions-rate measurements.* – Individual wood-piece $CO_2$ release rates were measured in the field in a closed, ventilated chamber (10 L) connected to an infrared gas analyzer (Licor 820, Lincoln, NE, USA). After chamber closure and initial stabilization, linear $CO_2$ concentration increase rates were logged for at least 5 min. Pieces remained in the field for $CO_2$ measurements (within 5 m) and were handled carefully to limit fragmentation. Temperature and moisture were measured for each sample at each sampling time. The wood-piece $CO_2$ release rate ($R_{WD}$, μmol C $g^{-1}$ $h^{-1}$) was calculated as follows:

$$R_{WD} = (1000*\Delta CO_2*P*(V-V_s)) / (24*R*(T_s+273)*W_C) \quad (1)$$

where $\Delta CO_2$ represents the measured $CO_2$ concentration increase (ppm $day^{-1}$), P is the internal pressure (kPa; measured by the Licor 820), V is the volume of the system (10.08 L, including the chamber volume and tubing volume and Licor optical path), $V_s$ is the volume of the wood piece (L), R is the gas constant (8.314 $L^{-1}$ $kPa^{-1}$ $K^{-1}$ $mol^{-1}$), $T_s$ is the wood temperature (ºC), and WC is the carbon weight of each piece (g; 47% of its dry weight).

These measurements were made eight times from September 2010 to June 2013, approximately every four months (Fig. 1).

*Sampling for genetic analysis.* – The larger ongoing study includes three species, each having three decay classes, thus producing nine strata. 65 wood pieces were selected from all nine strata. In addition, for each stratum, we calculated a mean $CO_2$ emission rate during 2010-2012, and the pieces chosen for this experiment included ones that were consistently below, at, or above the mean rate, and also some that showed variation around the mean over time. Pieces for each of these subgroups were selected at random from the larger study. This

288 stratified random sampling ensured that all nine groups and the full range of $CO_2$ emission
289 variation were represented in the metabarcoded samples. Those wood pieces were collected
290 in September 2012, and 59 of those samples were recollected in June 2013 (6 samples were
291 not relocated in June 2013, having lost their tags or been buried under new litter). We chose
292 June and September because $CO_2$ emissions from wood are typically higher in June and
293 lower in September, despite similarly warm temperatures and high moisture availability[8] (Fig.
294 1 top). Superficial litter and bark were removed from each wood piece with a flame-sterilized
295 knife before drilling. An electric drill with a flame-sterilized, 11-mm drill bit was used to
296 extract wood powder at three holes located near the ends and the middle of each wood piece.
297 Each sample consisted of ~5 $cm^3$ pooled material from those three holes, collected onto
298 aluminum-foil sheets, and then immediately stored in 50 ml tubes and frozen at -20 ºC for 2-3
299 days until transport on ice packs to our laboratory 10 hr away, where they were stored at -40
300 ºC until extractions.

301 *Comparing cumulative $CO_2$ emissions and gravimetric weight losses.* – Sixty-four
302 additional wood pieces (*i.e.* not used for metabarcoding) were retrieved from the field in
303 April 2013 to test the extent to which $CO_2$-emissions-estimated mass loss (averaged over the
304 measurements taken from the year 2010 experiment start) accurately estimated directly
305 measured mass loss (gravimetric weight loss). In the laboratory, these wood pieces were
306 re-measured for volume (as above), and twenty-two wood pieces exhibiting >15% volumetric
307 weight loss since the start of the experiment, indicating substantial fragmentation in the field,
308 were excluded from the analysis. The remaining wood pieces were dried at 70 ºC to constant
309 weights and then individually weighed to the nearest 0.1 g on an electronic balance.
310 $CO_2$-estimated mass loss was positively correlated with gravimetric loss (linear regression,
311 Gravimetric loss in grams over 3 years = 30.7 + 0.741 * $CO_2$-based-decomposition Cd ($R^2$ =
312 0.769, n = 17, p <0.01); 28.7 + 0.56 * Cd ($R^2$ = 0.762, n = 13, p <0.01); and 40.3 + 0.468 *
313 Cd ($R^2$ = 0.592, n = 13, p <0.01), for DKC1, 2, and 3, respectively. Inspection of scatterplots
314 (Supporting Information S5) revealed that the main discrepancy was that $CO_2$-emission
315 mass-loss estimates slightly underestimated small mass losses.

316 *DNA extraction, PCR, and 454 pyrosequencing of ITS2 amplicons.* – Total DNA was
317 extracted from each sample of wood powder by adding 10 mL CTAB buffer (2% cetyl

318 trimethyl ammonium bromide, 50 mM NaCl, 5 mM EDTA, 10 mM Tris, pH 8) and 20 μL
319 β-mercaptoethanol per 5 $cm^3$ of sample, homogenizing with a TH-02 homogenizer (Omni
320 International, Kennesaw, GA USA) for 5 min at room temperature, incubating at 65 ºC for 1
321 hour, centrifuging at 4,000 rpm for 1 min. After centrifugation, the supernatant was
322 transferred to new Axygen® 2.0 mL microcentrifuge tubes and extracted using one volume of
323 chloroform by vortexing for 20 min and centrifuged at 4 ºC, 12,000 rpm for 10 min. The
324 supernatant was then transferred to new microcentrifuge tubes and precipitated with 1.5
325 volumes of precooled isopropanol at -20 ºC overnight. After centrifugation at 4 ºC, 12,000
326 rpm for 20 min, the precipitate was washed with 70% ethanol and dissolved in 100 μL TE
327 buffer (10 mM Tris, 1 mM EDTA, pH 8.0). DNA was purified by using QIAquick PCR
328 Purification Kit. The quantity and quality of purified DNA was assessed with a Nanodrop
329 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). Samples were
330 PCR amplified using the forward primer ITS3_KYO2 5'-GATGAAGAACGYAGYRAA-3' [18]
331 and reverse primer ITS4 5'-TCCTCCGCTTATTGATATGC-3' [53]. The standard Roche
332 A-adaptor and a unique 10 bp MID (Multiplex Identifier) tag for each sample were attached
333 to the forward primer. PCRs were performed using approximately 10 ng DNA in a 20 μL
334 reaction mixture containing 2 μL of 10X buffer ($Mg^{2+}$ Plus), 0.02 mM dNTPs, 20 μg Bovine
335 Serum Albumin, 1 μL DMSO, 0.4 μM of each primer, and 0.5 U HotStart Taq DNA
336 polymerase (TaKaRa Biotechnology Co., Dalian, China) under a temperature profile of 95 ºC
337 for 10 min, followed by 35 cycles of 94 ºC for 20 sec, 47 ºC for 30 sec, and 72 ºC for 2 min,
338 and final extension at 72 ºC for 7 min. For pyrosequencing, PCR products were gel-purified
339 using Qiagen QIAquick PCR purification kit, quantified using the Quant-iT PicoGreen
340 dsDNA Assay kit (Invitrogen, Grand Island, NY, USA), pooled and A-amplicon-sequenced
341 on a Roche GS FLX (Branford, Connecticut, USA) at the Kunming Institute of Zoology.
342 *Bioinformatic analyses.* – The sequences obtained were run through a pipeline for quality
343 control, denoising and chimera removal, OTU-picking and taxonomic assignment. Quality
344 Control: Header sequences and low-quality reads were removed from the raw output in the
345 QIIME 1.8.0 environment (*split_libraries.py*: -l 100 -L 500 -H 30)[54]. The 65 samples
346 collected in 2012 had been sequenced on four 1/8 regions, producing 679,361 raw reads and
347 525,679 post-quality-control reads (mean read length 286 bp). The 59 samples collected in

348 2013 had been sequenced on three 1/8 regions, producing 327,226 raw reads and 256,996
349 post-quality-control reads (mean read length 308 bp). These amplicon lengths were consistent
350 with the expected mean length for ITS2 (327.2 bp, SD=40) reported by Toju *et al*.[18].
351 *Denoising and chimera removal: Denoiser* in QIIME[55] was used to remove characteristic 454
352 sequencing errors. Next, ITSx 1.0.3[56] was used to extract the variable ITS2 region from the
353 whole reads (i.e. conserved 5.8S and LSU flanking sequences were stripped) and to remove
354 non-fungal-ITS reads. The extracted sequences were clustered at 99% similarity with
355 USEARCH v7.0.1090[57] to remove replicate sequences and chimeras. *OTU-picking and*
356 *taxonomic assignment*: We used two methods to cluster the reads into OTUs. First, we used a
357 reference-based method in QIIME (*pick_open_reference_otus.py*: max_accepts 20
358 max_rejects 500 stepwords 20 length 12 –suppress_align_and_tree) in which reads were first
359 clustered by matching at 97% similarity to the UNITE 12_11 fungal database[58], which itself
360 had previously been clustered at 97% similarity for use within QIIME. Unassigned reads (the
361 vast majority) were then clustered *de novo* using the *uclust* option at 97% similarity,
362 producing 1,807 OTUs in total. For these latter OTUs, we attempted to assign taxonomies
363 using QIIME's *assign_taxonomy.py* against the UNITE database. Second, we performed *de*
364 *novo* 97%-similarity clustering with CROP 1.33[59], producing 1,565 OTUs. We assigned
365 taxonomies against Genbank using the NNCauto and QCauto methods in Claident[60].
366 Sequence data are deposited at datadryad.org (doi: to be assigned) and in GENBANK's Short
367 Read Archive (Accession number: PRJNA252416). An example bioinformatic script is in
368 Supplementary Information and also deposited at datadryad.org (doi: to be assigned).
369 *Statistical analyses.* – Analyses were performed using *vegan* 2.0-10[61] and *mvabund*
370 3.8.4[62] in *R* 3.1.0[63]. The HTML outputs of the R scripts are in Supplementary Information and
371 also deposited at datadryad.org (doi: to be assigned). From both the OTU tables generated
372 (CROP and *uclust*, see *Bioinformatic analysis*), we deleted one SN wood piece that had only
373 35 reads and then split the tables by wood species (LC, LX, and SN) and sample time
374 (September 2012 and June 2013). We then generated a second pair of OTU tables by
375 rarefying to the lowest read number per wood piece in the dataset (*rrarefy()* in *R*). Thus, for
376 each wood species and sampling time, we have four OTU tables: CROP/non-rarefied,
377 CROP/rarefied, *uclust*/non-rarefied, and *uclust*/rarefied. Finally, for each table, we used

*vegan*'s *diversity()* function to estimate Shannon and Simpson diversity in each wood piece.

For each wood species, we used *lm()* in *R* to linearly regress emissions against fungal diversity. Thus, the June 2012 and September 2012 $CO_2$ estimates were tested against the September 2012 fungal diversity estimate, and the June 2013 $CO_2$ estimate was tested against the June 2013 fungal diversity estimate. Residuals were all adjudged visually to be near normally distributed, but with small indications of nonlinearity. We ignored the nonlinearities because the residuals suggested accelerating $CO_2$ emissions at the lowest fungal diversity, making our results conservative. In trial models, we also tested for significant effects of wood surface temperature and decay class (see *Experimental setup*), but they did not interact significantly with the fungal diversity term and mostly did not enter significantly as additive terms, and so have been omitted here for simplicity. Liu *et al*.[8] also did not find a correlation between decay class and $CO_2$ emissions rates for these wood pieces.

To test whether a 'pure diversity' effect is sufficient to explain the observed diversity-function relationships, we use a method by Sandau *et al*.[24] where community similarities (1-Jaccard binary) between all wood pieces are used to create a variance-covariance matrix that is then included in the linear regressions, thus taking into account potential non-independence of wood pieces due to the fact that some communities are similar to each other (Supporting Information S3). In Supporting Information S4, we also use conventional community analyses to test for an effect of community composition on $CO_2$ emissions. We limit our tests to June 2012 and 2013, as only these exhibited significant declines in emissions with fungal-species diversity (Fig. 1A, C, D, F).

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## Author contributions

DAS, CYY, and DWY designed the study. CYY, WJL and DAS carried out the field work. CYY, CXY, XYW and CYW performed the DNA analyses. CYY and DWY carried out the bioinformatics. DWY and VP carried out the statistical analyses. DWY, DAS and CYY wrote the first draft. WJL and CXY commented on the manuscript.

## Additional information

DWY is co-founder of a UK company, NatureMetrics, which provides DNA metabarcoding services to the private and public sector. All other authors declare no conflicts of interest.

575 ## Figure legends

576 **Figure 1**. Linear regressions of $CO_2$ emission rates on Shannon fungal diversities measured
577 from individually metabarcoded wood pieces. **Top**. The solid black curve indicates the air
578 temperature. Carets indicate times of $CO_2$ measurements. Blue shading indicates the warm
579 months when wood decomposition is >50% of maximum. **A-C**. $CO_2$ emissions decline with
580 increased fungal species diversity in two of the species in June 2012 (LC and SN) and in all
581 three species in June 2013. In September 2012, $CO_2$ emissions are lower, and there is no
582 relationship. The OTU-picking method is *de novo* clustering with CROP. **D-F**. Same as A-C
583 but the OTU-picking method is QIIME's reference-based matching against the UNITE
584 database, with *de novo* clustering of non-matched reads with *uclust*. Non-significant
585 regressions are indicated by dashed lines. Shown here are the non-rarefied datasets.
586 Rarefaction does not change the results (Supporting Information S1). LC = *Lithocarpus*
587 *chintungensis,* LX = *L. xylocarpus*, SN = *Schima noronhae*.

588 **Figure 2.** Correspondence analysis ordinations of fungal communities, by tree species and
589 sampling date. Point size is scaled to $CO_2$ emissions, and the gradient represents fungal
590 Shannon diversity. In all ordinations (**A-F**), $CO_2$ emissions decrease with higher fungal
591 diversity (point size decreases up the gradient, echoing Fig. 1). Also evident is that the lower
592 diversity wood pieces are compositionally very dissimilar to each other and to the higher
593 diversity wood pieces. **Left-hand column (A, C, E).** June 2012 $CO_2$ vs. September 2012
594 fungal diversity. **Right-hand column (B, D, F).** June 2013 $CO_2$ vs. June 2013 fungal
595 diversity. **A-B**. *Lithocarpus chintungensis*. Note that the label for point 14 at the top of A is
596 obscured by the small point size. **C-D.** *L. xylocarpus*. **E-F.** *Schima noronhae*. Shown here are
597 the non-rarefied datasets clustered using CROP (see Methods). Rarefaction or using
598 *uclust*-clustering does not change the results (Supporting Information S4).

## Tables

**Table 1**. Taxonomic assignments to Class level for the ITS2 Operational Taxonomic Units (OTUs). *uclust* and CROP refer to the two OTU-clustering methods used, and GenBank and UNITE refer to the fungal reference databases used (see Methods: *Bioinformatic Analyses* for details).

| CROP + Genbank (1,565 OTUs) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Kingdom Fungi | Ascomycota | Basidiomycota | Chytridiomycota | Zygomycota | Glomeromycota | Mortierellales | Phylum unidentified |
| 1194 OTUs | 632 | 319 | 1 | 3 | 1 | 3 | 235 |
| 76.3% of total | 52.9% of Fungi | 26.70% | 0.10% | 0.30% | 0.10% | 0.30% | 19.70% |
| *uclust* + UNITE (1,806 OTUs) | | | | | | | |
| Kingdom Fungi | Ascomycota | Basidiomycota | Chytridiomycota | Zygomycota | Phylum unidentified | | |
| 742 OTUs | 378 | 169 | 2 | 5 | 188 | | |
| 41.1% of total | 50.9% of Fungi | 22.80% | 0.30% | 0.70% | 25.30% | | |

**Table 2**. Estimates of the contribution of fungal community composition to $CO_2$ emissions, using the method of Sandau *et al*.[24]. The generated parameter λ varies between 0 and 1, with 0 indicating that variation in composition does not explain variation in emissions. Composition only contributes to explaining variation in one tree species in one sampling date (SN, June 2013). Conventional community analysis (Supporting Information S4) also detected a contribution of composition in SN in June 2013. LC = *Lithocarpus chintungensis,* LX = *L. xylocarpus*, SN = *Schima noronhae*.

| $CO_2$ sample | Species | OTU clustering | λ |
|---|---|---|---|
| Jun-12 | LC | CROP | 0.000 |
| | | *uclust* | 0.000 |
| | LX | CROP | 0.002 |
| | | *uclust* | 0.000 |
| | SN | CROP | 0.001 |
| | | *uclust* | 0.004 |
| Jun-13 | LC | CROP | 0.000 |
| | | *uclust* | 0.000 |
| | LX | CROP | 0.000 |
| | | *uclust* | 0.001 |
| | SN | CROP | 0.366 |
| | | *uclust* | 0.500 |

# Higher fungal diversity is correlated with lower $CO_2$ emissions from dead wood in a natural forest: <u>Supplementary Information</u>

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# Higher fungal diversity is correlated with lower $CO_2$ emissions from dead wood in a natural forest:

## S1 Summary of Regression Analyses

**S1 Summary of Regression analyses**

| OTU-pickng | rarefaction | CO2 measurement | species | diversity | p-value | notes |
|---|---|---|---|---|---|---|
| CROP | nonrarefied | Jun-12 | LC | Shannon | **0.007** | |
| CROP | nonrarefied | Jun-12 | LC | Simpson | 0.015 | |
| CROP | nonrarefied | Jun-12 | LX | Shannon | **0.080** | |
| CROP | nonrarefied | Jun-12 | LX | Simpson | 0.040 | |
| CROP | nonrarefied | Jun-12 | SN | Shannon | **0.027** | removed influential woodpiece=110 |
| CROP | nonrarefied | Jun-12 | SN | Simpson | 0.018 | removed influential woodpiece=110 |
| CROP | nonrarefied | Sep-12 | LC | Shannon | **0.460** | |
| CROP | nonrarefied | Sep-12 | LC | Simpson | 0.642 | |
| CROP | nonrarefied | Sep-12 | LX | Shannon | **0.116** | |
| CROP | nonrarefied | Sep-12 | LX | Simpson | 0.057 | |
| CROP | nonrarefied | Sep-12 | SN | Shannon | **0.700** | |
| CROP | nonrarefied | Sep-12 | SN | Simpson | 0.474 | |
| CROP | nonrarefied | Jun-13 | LC | Shannon | **0.003** | |
| CROP | nonrarefied | Jun-13 | LC | Simpson | 0.000 | |
| CROP | nonrarefied | Jun-13 | LX | Shannon | **0.025** | |
| CROP | nonrarefied | Jun-13 | LX | Simpson | 0.016 | |
| CROP | nonrarefied | Jun-13 | SN | Shannon | **0.003** | |
| CROP | nonrarefied | Jun-13 | SN | Simpson | 0.001 | |
| CROP | rarefied | Jun-12 | LC | Shannon | 0.005 | |
| CROP | rarefied | Jun-12 | LC | Simpson | 0.014 | |
| CROP | rarefied | Jun-12 | LX | Shannon | 0.084 | |
| CROP | rarefied | Jun-12 | LX | Simpson | 0.044 | |
| CROP | rarefied | Jun-12 | SN | Shannon | 0.020 | removed influential woodpiece=110 |
| CROP | rarefied | Jun-12 | SN | Simpson | 0.037 | removed influential woodpiece=110 |
| CROP | rarefied | Sep-12 | LC | Shannon | 0.563 | |
| CROP | rarefied | Sep-12 | LC | Simpson | 0.572 | |

The (bolded) Shannon results from this first section are plotted in Fig. 1A-C.

| | | | | | | |
|---|---|---|---|---|---|---|
| CROP | rarefied | Sep-12 | LX | Shannon | 0.123 | |
| CROP | rarefied | Sep-12 | LX | Simpson | 0.062 | |
| CROP | rarefied | Sep-12 | SN | Shannon | 0.693 | |
| CROP | rarefied | Sep-12 | SN | Simpson | 0.475 | |
| CROP | rarefied | Jun-13 | LC | Shannon | 0.004 | |
| CROP | rarefied | Jun-13 | LC | Simpson | 0.001 | |
| CROP | rarefied | Jun-13 | LX | Shannon | 0.030 | |
| CROP | rarefied | Jun-13 | LX | Simpson | 0.019 | |
| CROP | rarefied | Jun-13 | SN | Shannon | 0.004 | |
| CROP | rarefied | Jun-13 | SN | Simpson | 0.001 | |
| uclust | nonrarefied | Jun-12 | LC | Shannon | **0.003** | |
| uclust | nonrarefied | Jun-12 | LC | Simpson | 0.005 | |
| uclust | nonrarefied | Jun-12 | LX | Shannon | **0.086** | |
| uclust | nonrarefied | Jun-12 | LX | Simpson | 0.044 | |
| uclust | nonrarefied | Jun-12 | SN | Shannon | **0.538** | removed influential woodpiece=110 |
| uclust | nonrarefied | Jun-12 | SN | Simpson | 0.439 | removed influential woodpiece=110 |
| uclust | nonrarefied | Sep-12 | LC | Shannon | **0.499** | |
| uclust | nonrarefied | Sep-12 | LC | Simpson | 0.593 | |
| uclust | nonrarefied | Sep-12 | LX | Shannon | **0.138** | |
| uclust | nonrarefied | Sep-12 | LX | Simpson | 0.066 | |
| uclust | nonrarefied | Sep-12 | SN | Shannon | **0.500** | |
| uclust | nonrarefied | Sep-12 | SN | Simpson | 0.265 | |
| uclust | nonrarefied | Jun-13 | LC | Shannon | **0.003** | |
| uclust | nonrarefied | Jun-13 | LC | Simpson | 0.000 | |
| uclust | nonrarefied | Jun-13 | LX | Shannon | **0.028** | |
| uclust | nonrarefied | Jun-13 | LX | Simpson | 0.018 | |
| uclust | nonrarefied | Jun-13 | SN | Shannon | **0.004** | |
| uclust | nonrarefied | Jun-13 | SN | Simpson | 0.001 | |

The (bolded) Shannon results from this thrd section are plotted in Fig. 1D-F.

| | | | | | | |
|---|---|---|---|---|---|---|
| uclust | rarefied | Jun-12 | LC | Shannon | 0.005 | |
| uclust | rarefied | Jun-12 | LC | Simpson | 0.003 | |
| uclust | rarefied | Jun-12 | LX | Shannon | 0.094 | |
| uclust | rarefied | Jun-12 | LX | Simpson | 0.049 | |
| uclust | rarefied | Jun-12 | SN | Shannon | 0.096 | removed influential woodpiece=110 |
| uclust | rarefied | Jun-12 | SN | Simpson | 0.144 | removed influential woodpiece=110 |
| uclust | rarefied | Sep-12 | LC | Shannon | 0.441 | |
| uclust | rarefied | Sep-12 | LC | Simpson | 0.590 | |
| uclust | rarefied | Sep-12 | LX | Shannon | 0.136 | |
| uclust | rarefied | Sep-12 | LX | Simpson | 0.066 | |
| uclust | rarefied | Sep-12 | SN | Shannon | 0.482 | |
| uclust | rarefied | Sep-12 | SN | Simpson | 0.247 | |
| uclust | rarefied | Jun-13 | LC | Shannon | 0.004 | |
| uclust | rarefied | Jun-13 | LC | Simpson | 0.001 | |
| uclust | rarefied | Jun-13 | LX | Shannon | 0.026 | |
| uclust | rarefied | Jun-13 | LX | Simpson | 0.018 | |
| uclust | rarefied | Jun-13 | SN | Shannon | 0.004 | |
| uclust | rarefied | Jun-13 | SN | Simpson | 0.001 | |

# Higher fungal diversity is correlated with lower $CO_2$ emissions from dead wood in a natural forest:

## S2 Wood chemistry analyses

**Supporting Information S2**. **Wood chemistry analyses.**

## Methods

In January 2013, 27 decaying wood samples from our larger study were removed from the field for chemical analyses. Included were 3 pieces from each combination of wood species (LC, LX, and SN) and decay class (DKC 1, 2, 3), spanning the range of previously observed decomposition rates. These pieces were dried at 60 $^{o}$C to constant weights and milled to pass a 40 mesh (425 micron opening) sieve. They were analyzed for total carbon and nitrogen (Macro Elemental CN Analyzer, Vario MAX CN, Germany), for total phosphorus (continuous Flow Analyzer, Auto Analyzer 3, Germany) after acid digestion, and for lignin (Fibertec 2010 System, Fibertec, Denmark). The metabarcoded wood pieces remain in the field and were not analyzed.

## Column headings explanation

Tag: Permanent identification number assigned to each piece of wood in this study

Tree_species: LC = *Lithocarpus chintungensis*, LX = *Lithocarpus xylocarpus* (LX), and SN = *Schima noronhae*

Average_$CO_2$: $CO_2$ release rate for each wood piece from 2010 to 2012, average of 6 measurements, micromoles $CO_2$ per dry gram of wood carbon per hour

Total_carbon_g/kg: Carbon as grams per dry kilogram of wood weight

Total_Nitrogen_g/kg: Nitrogen as grams per dry kilogram of wood weight

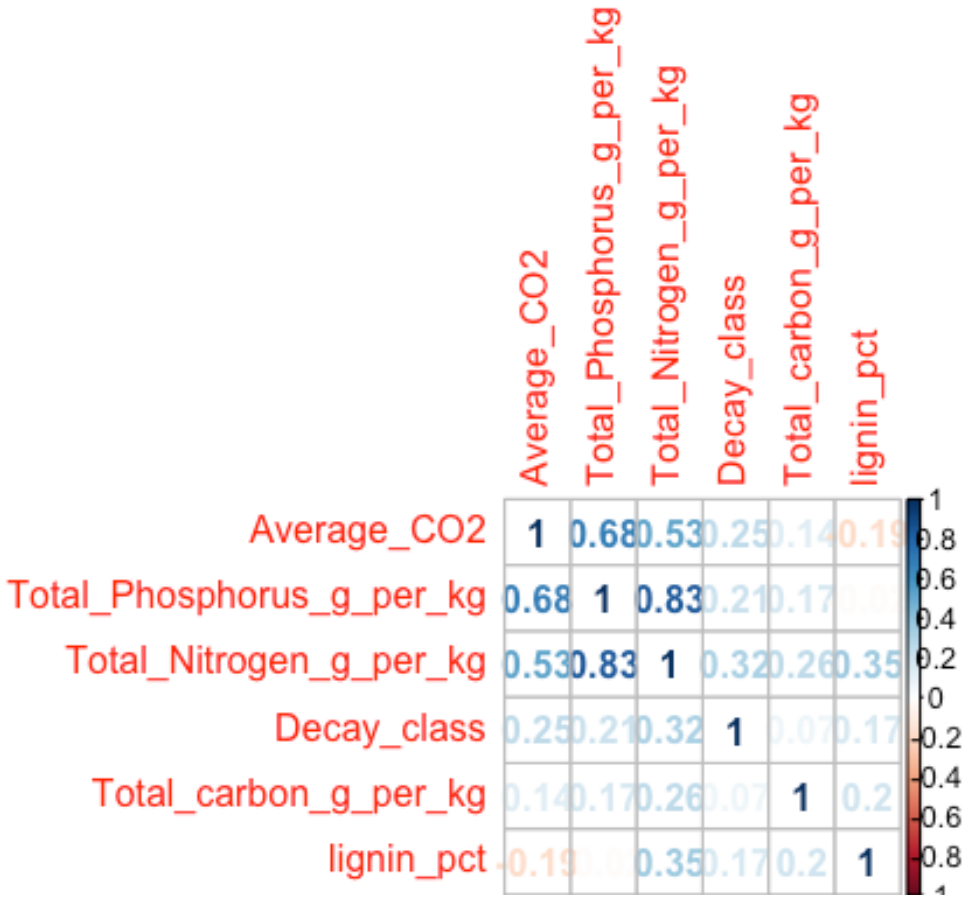
Total_Phosphorous_g/kg: Phosphorus as grams per dry kilogram of wood weight

lignin_%: Lignin as percent of dry wood-piece weight

| Tag | Tree_species | Decay_class | Average_$CO_2$ | Total_carbon_g/kg | Total_Nitrogen_g/kg | Total_Phosphorus_g/kg | lignin_% |
|---|---|---|---|---|---|---|---|
| 52 | LC | 1 | 0.529 | 458 | 3.96 | 0.20 | 26.8 |
| 160 | LC | 1 | 0.482 | 452 | 3.7 | 0.16 | 19.6 |
| 164 | LC | 1 | 0.335 | 455 | 3.51 | 0.12 | 19.7 |
| 2 | LC | 2 | 0.601 | 450 | 3.67 | 0.17 | 22.1 |
| 119 | LC | 2 | 1.980 | 496 | 4.30 | 0.22 | 18.6 |
| 214 | LC | 2 | 0.298 | 460 | 2.65 | 0.07 | 16.4 |
| 11 | LC | 3 | 0.450 | 458 | 3.08 | 0.13 | 23.7 |
| 67 | LC | 3 | 0.723 | 488 | 7.56 | 0.27 | 31.0 |
| 76 | LC | 3 | 0.404 | 491 | 4.69 | 0.11 | 25.2 |
| 92 | LX | 1 | 0.264 | 497 | 2.69 | 0.08 | 23.0 |
| 219 | LX | 1 | 0.358 | 454 | 3.33 | 0.20 | 19.3 |
| 223 | LX | 1 | 0.796 | 455 | 4.19 | 0.25 | 17.8 |
| 141 | LX | 2 | 1.146 | 492 | 5.78 | 0.36 | 16.0 |
| 142 | LX | 2 | 1.430 | 505 | 8.38 | 0.64 | 19.4 |
| 243 | LX | 2 | 0.392 | 454 | 3.44 | 0.11 | 24.8 |
| 191 | LX | 3 | 1.493 | 452 | 5.42 | 0.57 | 17.3 |
| 267 | LX | 3 | 1.454 | 459 | 7.40 | 0.37 | 24.0 |
| 269 | LX | 3 | 0.534 | 464 | 5.47 | 0.27 | 18.7 |
| 29 | SN | 1 | 0.269 | 455 | 2.29 | 0.06 | 16.7 |
| 40 | SN | 1 | 0.752 | 451 | 3.92 | 0.17 | 20.8 |
| 317 | SN | 1 | 0.463 | 527 | 2.42 | 0.12 | 20.6 |
| 7 | SN | 2 | 0.803 | 454 | 3.17 | 0.13 | 19.3 |
| 108 | SN | 2 | 0.980 | 453 | 1.45 | 0.07 | 16.2 |
| 313 | SN | 2 | 0.421 | 453 | 3.11 | 0.09 | 18.4 |
| 23 | SN | 3 | 0.449 | 470 | 3.19 | 0.14 | 19.2 |
| 25 | SN | 3 | 0.848 | 452 | 1.88 | 0.07 | 16.6 |
| 321 | SN | 3 | 0.335 | 503 | 3.13 | 0.10 | 21.8 |

# Wood Chemistry: Supplementary Information S2

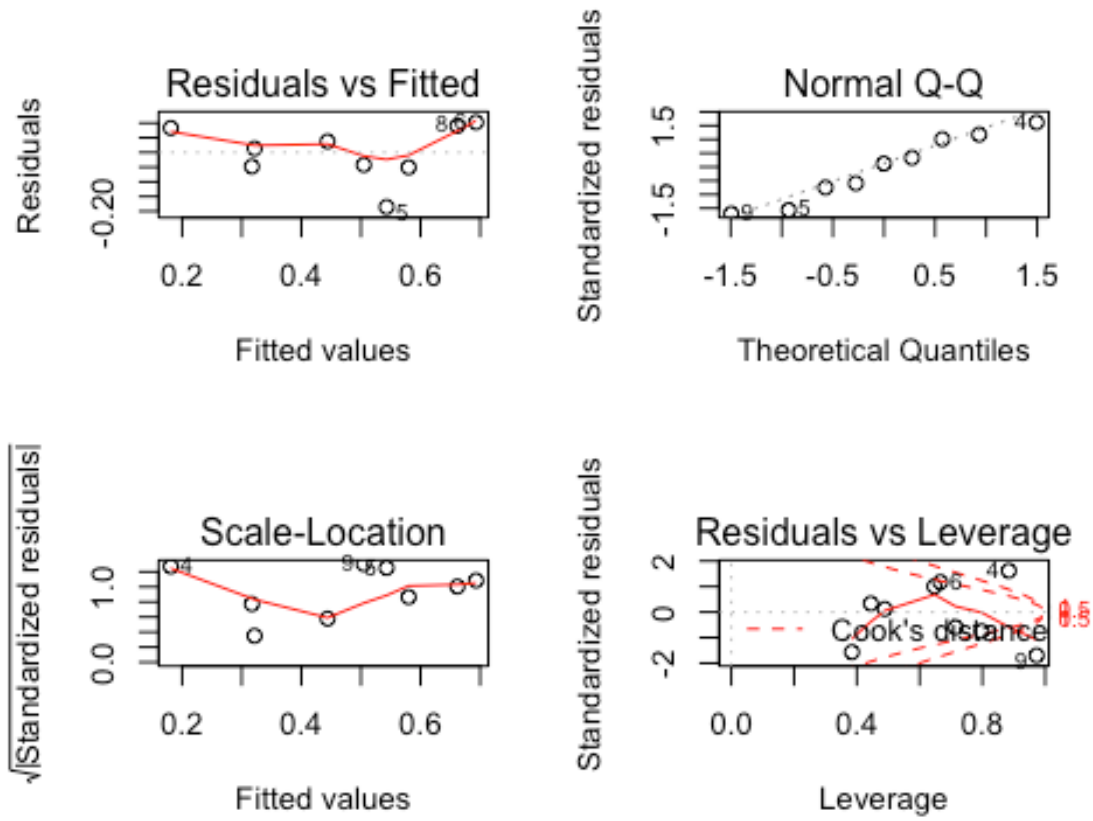
```
## [1] "Tag"                        "Tree_species"
## [3] "Decay_class"                "Average_CO2"
## [5] "Total_carbon_g_per_kg"      "Total_Nitrogen_g_per_kg"
## [7] "Total_Phosphorus_g_per_kg" "lignin_pct"
```




The high correlation between Total_Phosphorous_g_per_kg and Total_Nitrogen_g_per_kg means that we cannot include them in the same model (collinearity). So we run two separate models. We also run models separately for each decay class, because wood chemistry changes with age, due to selective retention of N and P while C is lost. This gives us 6 total models (3 decay classes X 2 models). As a result, we informally carry out a Table-wide correction of P-values by multiplying all P-values by 6 before assessing statistical significance.

## Decay class 1

```
wood_dkc1 <- wood %>% filter(Decay_class == 1)

mod1.1 <- lm(Average_CO2 ~ Tree_species + Total_carbon_g_per_kg + Total_Phosp
horus_g_per_kg + lignin_pct, data=wood_dkc1)
summary(mod1.1)

##
## Call:
## lm(formula = Average_CO2 ~ Tree_species + Total_carbon_g_per_kg +
##     Total_Phosphorus_g_per_kg + lignin_pct, data = wood_dkc1)
##
## Residuals:
##        1        2        3        4        5        6        7        8
## -0.05078  0.03754  0.01324  0.08299 -0.18550  0.10251 -0.04800  0.09029
##        9
## -0.04229
##
## Coefficients:
##                            Estimate Std. Error t value Pr(>|t|)
## (Intercept)               -6.173e-02  1.132e+00  -0.055   0.9599
## Tree_speciesLX            -2.262e-02  1.454e-01  -0.156   0.8863
## Tree_speciesSN             1.849e-01  1.553e-01   1.190   0.3195
## Total_carbon_g_per_kg     -4.838e-05  2.635e-03  -0.018   0.9865
## Total_Phosphorus_g_per_kg  3.061e+00  1.061e+00   2.884   0.0633 .
## lignin_pct                 1.936e-03  2.238e-02   0.087   0.9365
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1508 on 3 degrees of freedom
## Multiple R-squared:  0.775,  Adjusted R-squared:  0.3999
## F-statistic: 2.066 on 5 and 3 DF,  p-value: 0.2921

vif(mod1.1)

##                               GVIF Df GVIF^(1/(2*Df))
## Tree_species              1.929908  2        1.178648
## Total_carbon_g_per_kg     1.729095  1        1.314950
## Total_Phosphorus_g_per_kg 1.499699  1        1.224622
## lignin_pct                1.548025  1        1.244196

par(mfrow=c(2,2))
plot(mod1.1)
```




```
par(mfrow=c(1,1))
```

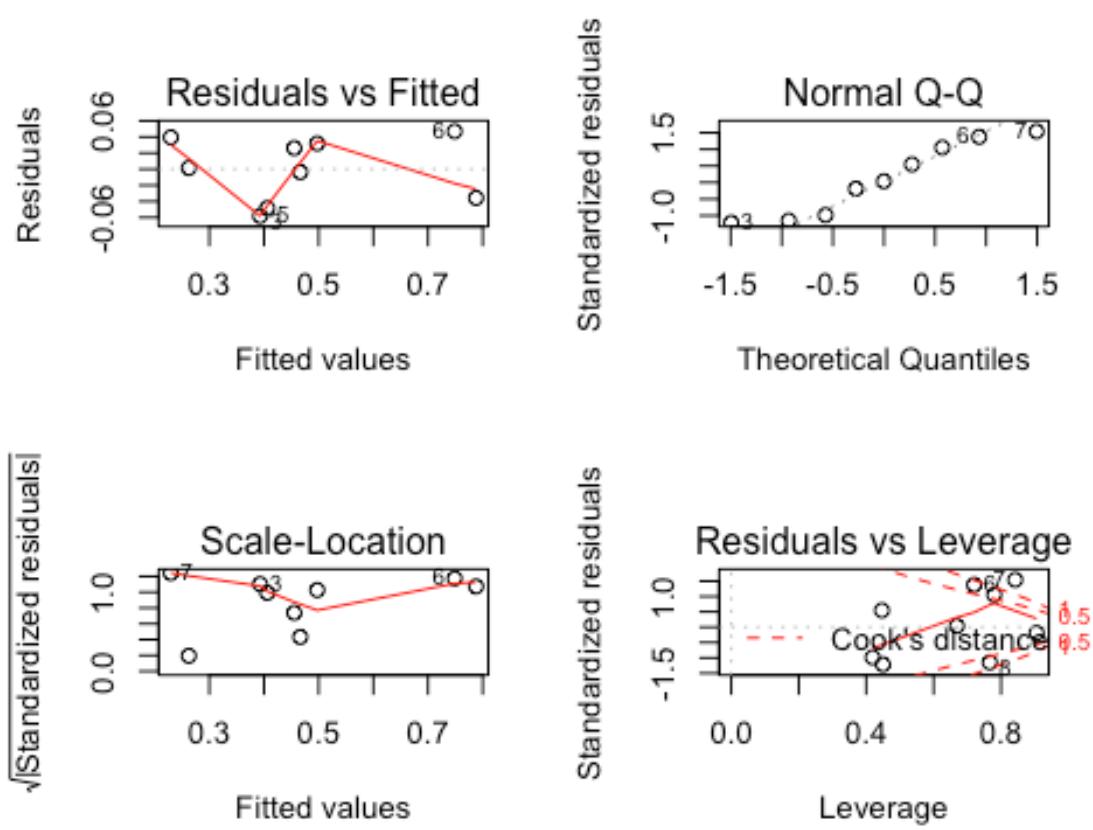
```
mod1.2 <- lm(Average_CO2 ~ Tree_species + Total_carbon_g_per_kg + Total_Nitro
gen_g_per_kg + lignin_pct, data=wood_dkc1)
summary(mod1.2)

##
## Call:
## lm(formula = Average_CO2 ~ Tree_species + Total_carbon_g_per_kg +
##     Total_Nitrogen_g_per_kg + lignin_pct, data = wood_dkc1)
##
## Residuals:
##         1         2         3         4         5         6         7
##  0.031887  0.026213 -0.058099  0.001404 -0.048399  0.046995  0.039522
##         8         9
## -0.035806 -0.003717
##
## Coefficients:
```

```
##                            Estimate Std. Error t value Pr(>|t|)
## (Intercept)               -2.171285   0.642439  -3.380   0.0431 *
## Tree_speciesLX             0.080254   0.059500   1.349   0.2702
## Tree_speciesSN             0.265343   0.067685   3.920   0.0295 *
## Total_carbon_g_per_kg      0.003182   0.001322   2.407   0.0953 .
## Total_Nitrogen_g_per_kg    0.376884   0.049140   7.670   0.0046 **
## lignin_pct                -0.010503   0.009978  -1.053   0.3698
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.06452 on 3 degrees of freedom
## Multiple R-squared:  0.9588, Adjusted R-squared:  0.8901
## F-statistic: 13.96 on 5 and 3 DF,  p-value: 0.02735
```

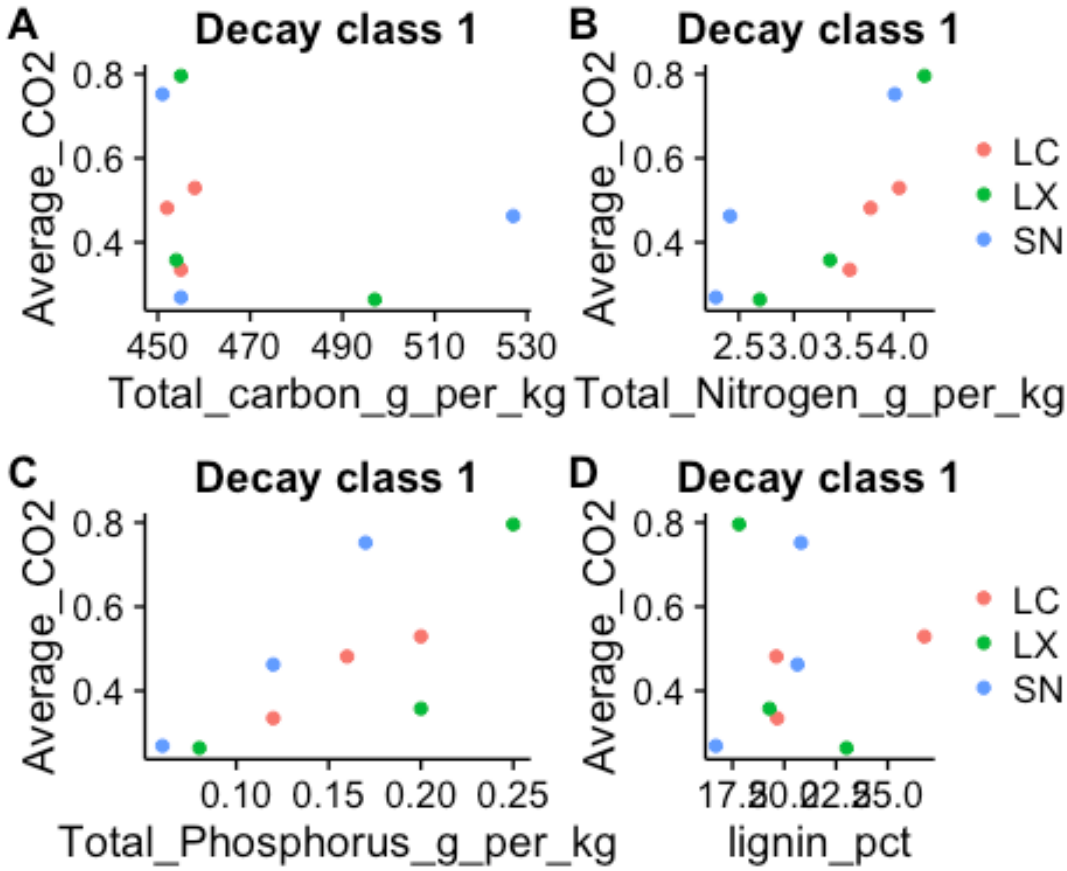
```r
vif(mod1.2)
```

```
##                              GVIF Df GVIF^(1/(2*Df))
## Tree_species             1.760129  2        1.151824
## Total_carbon_g_per_kg    2.376907  1        1.541722
## Total_Nitrogen_g_per_kg 2.304348  1        1.518008
## lignin_pct               1.680777  1        1.296448
```

```r
par(mfrow=c(2,2))
plot(mod1.2)
```



```r
par(mfrow=c(1,1))
```




## Decay class 2

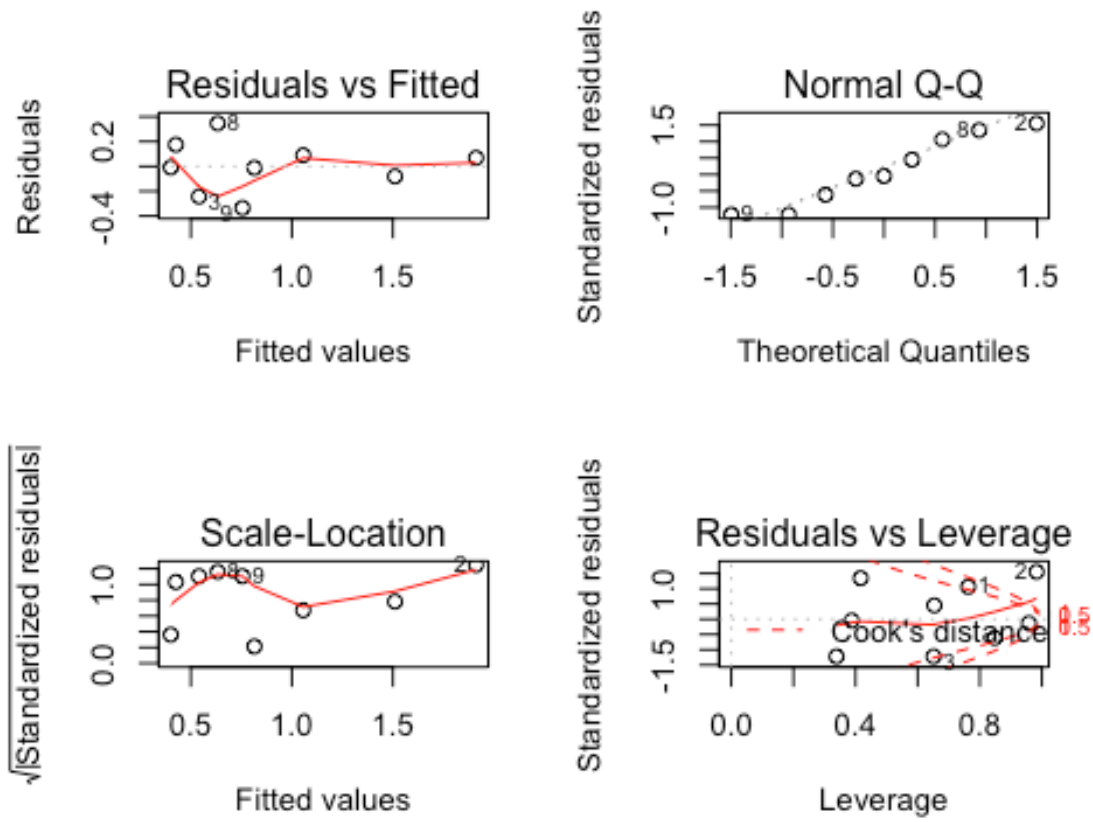
```r
wood_dkc2 <- wood %>% filter(Decay_class == 2)

mod2.1 <- lm(Average_CO2 ~ Tree_species + Total_carbon_g_per_kg + Total_Phosp
horus_g_per_kg + lignin_pct, data=wood_dkc2)
summary(mod2.1)

##
## Call:
## lm(formula = Average_CO2 ~ Tree_species + Total_carbon_g_per_kg +
##     Total_Phosphorus_g_per_kg + lignin_pct, data = wood_dkc2)
##
## Residuals:
##         1         2         3         4         5         6         7
##  0.174691  0.068453 -0.243143  0.089657 -0.080636 -0.009021 -0.011913
##         8         9
##  0.346874 -0.334961
##
## Coefficients:
##                            Estimate Std. Error t value Pr(>|t|)
## (Intercept)               -18.00200    6.55221  -2.747   0.0709 .
```

```
## Tree_speciesLX              -0.40482    0.35162  -1.151   0.3330
## Tree_speciesSN               0.37527    0.33322   1.126   0.3420
## Total_carbon_g_per_kg        0.03817    0.01294   2.949   0.0601 .
## Total_Phosphorus_g_per_kg   -0.93261    1.43524  -0.650   0.5621
## lignin_pct                   0.06381    0.05536   1.153   0.3325
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3374 on 3 degrees of freedom
## Multiple R-squared:  0.8613, Adjusted R-squared:  0.6301
## F-statistic: 3.726 on 5 and 3 DF,  p-value: 0.154
```

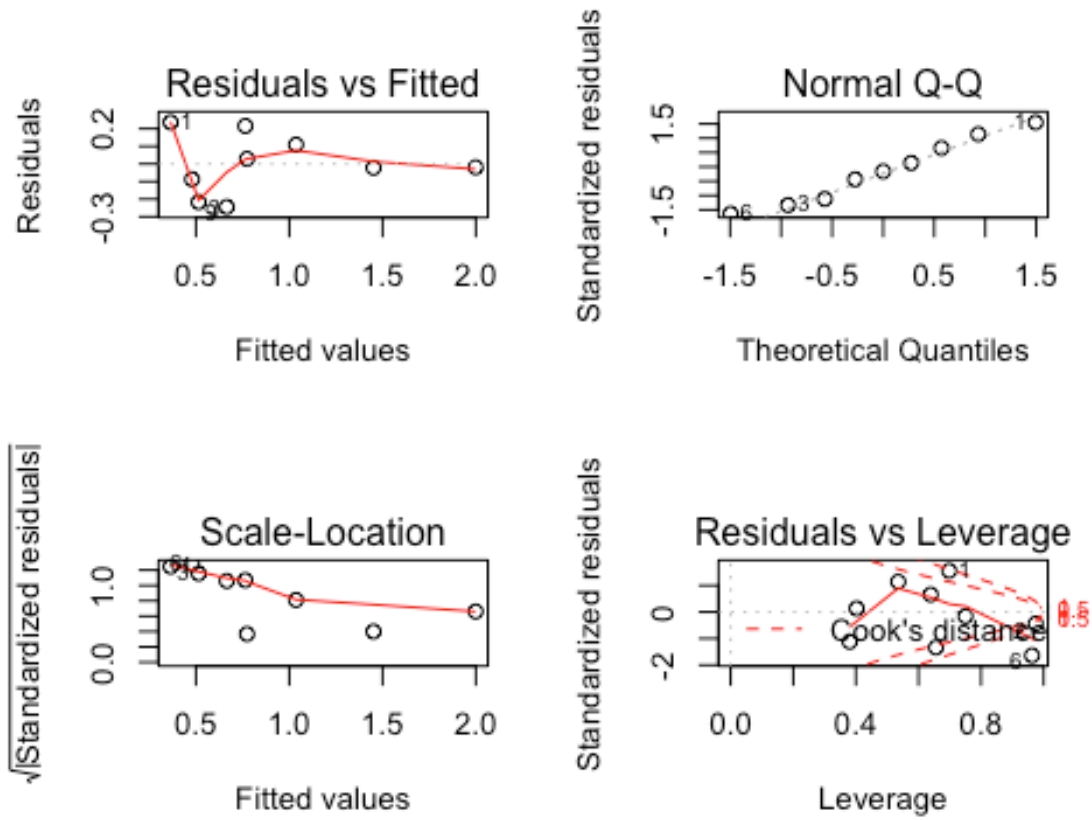
```r
vif(mod2.1)
```

```
##                                GVIF Df GVIF^(1/(2*Df))
## Tree_species               3.044532  2        1.320931
## Total_carbon_g_per_kg      5.819261  1        2.412314
## Total_Phosphorus_g_per_kg 5.047178  1        2.246592
## lignin_pct                 1.809937  1        1.345339
```

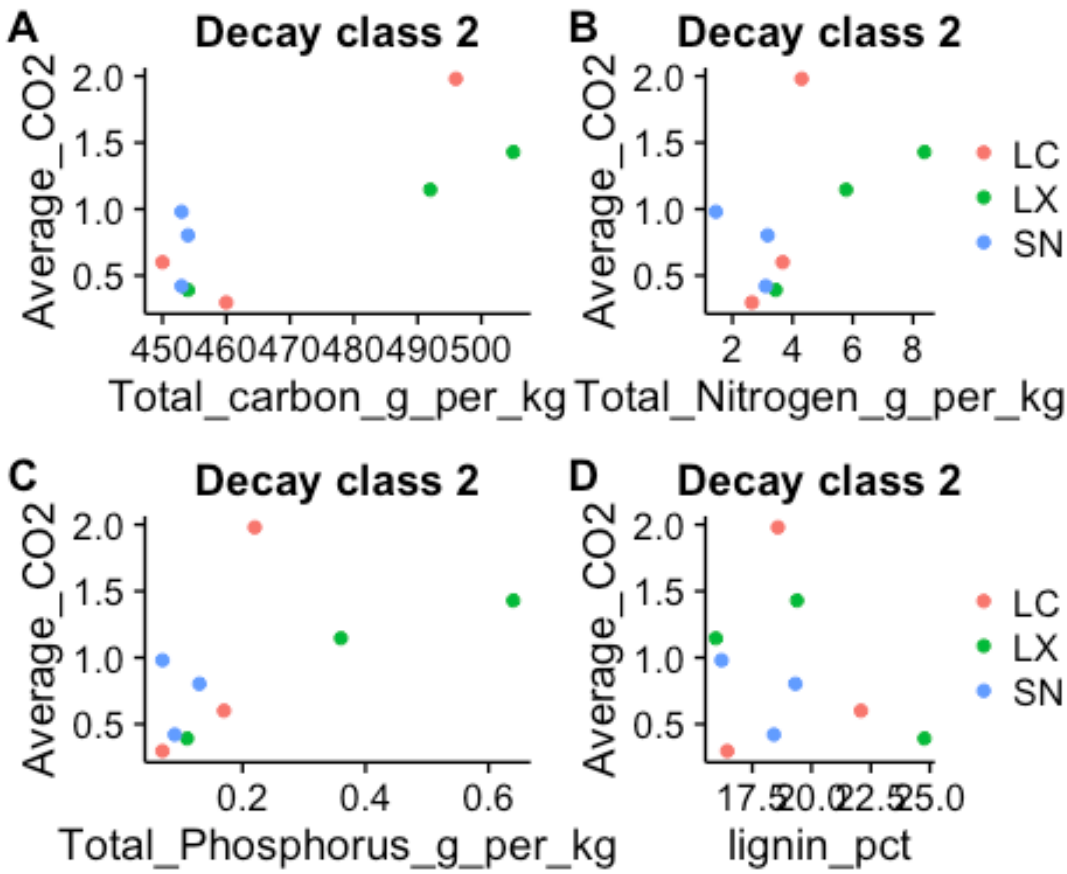
```r
par(mfrow=c(2,2))
plot(mod2.1)
```



```r
par(mfrow=c(1,1))

mod2.2 <- lm(Average_CO2 ~ Tree_species + Total_carbon_g_per_kg + Total_Nitro
gen_g_per_kg + lignin_pct, data=wood_dkc2)
summary(mod2.2)

##
## Call:
## lm(formula = Average_CO2 ~ Tree_species + Total_carbon_g_per_kg +
##     Total_Nitrogen_g_per_kg + lignin_pct, data = wood_dkc2)
##
## Residuals:
##        1        2        3        4        5        6        7        8
##  0.23634 -0.01925 -0.21709  0.10930 -0.02207 -0.08722  0.02891  0.21552
##        9
## -0.24442
##
## Coefficients:
##                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)             -20.67956    5.46283  -3.786   0.0323 *
## Tree_speciesLX           -0.32065    0.29045  -1.104   0.3502
## Tree_speciesSN            0.37561    0.26721   1.406   0.2545
## Total_carbon_g_per_kg     0.04412    0.01097   4.022   0.0276 *
## Total_Nitrogen_g_per_kg  -0.16922    0.11752  -1.440   0.2455
## lignin_pct                0.08208    0.04792   1.713   0.1853
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2771 on 3 degrees of freedom
## Multiple R-squared:  0.9064, Adjusted R-squared:  0.7505
## F-statistic: 5.813 on 5 and 3 DF,  p-value: 0.08912

vif(mod2.2)

##                             GVIF Df GVIF^(1/(2*Df))
## Tree_species            2.984744  2        1.314398
## Total_carbon_g_per_kg   6.196153  1        2.489207
## Total_Nitrogen_g_per_kg 5.874488  1        2.423734
## lignin_pct              2.010507  1        1.417923

par(mfrow=c(2,2))
plot(mod2.2)
```




```
par(mfrow=c(1,1))
```




## Decay class 3

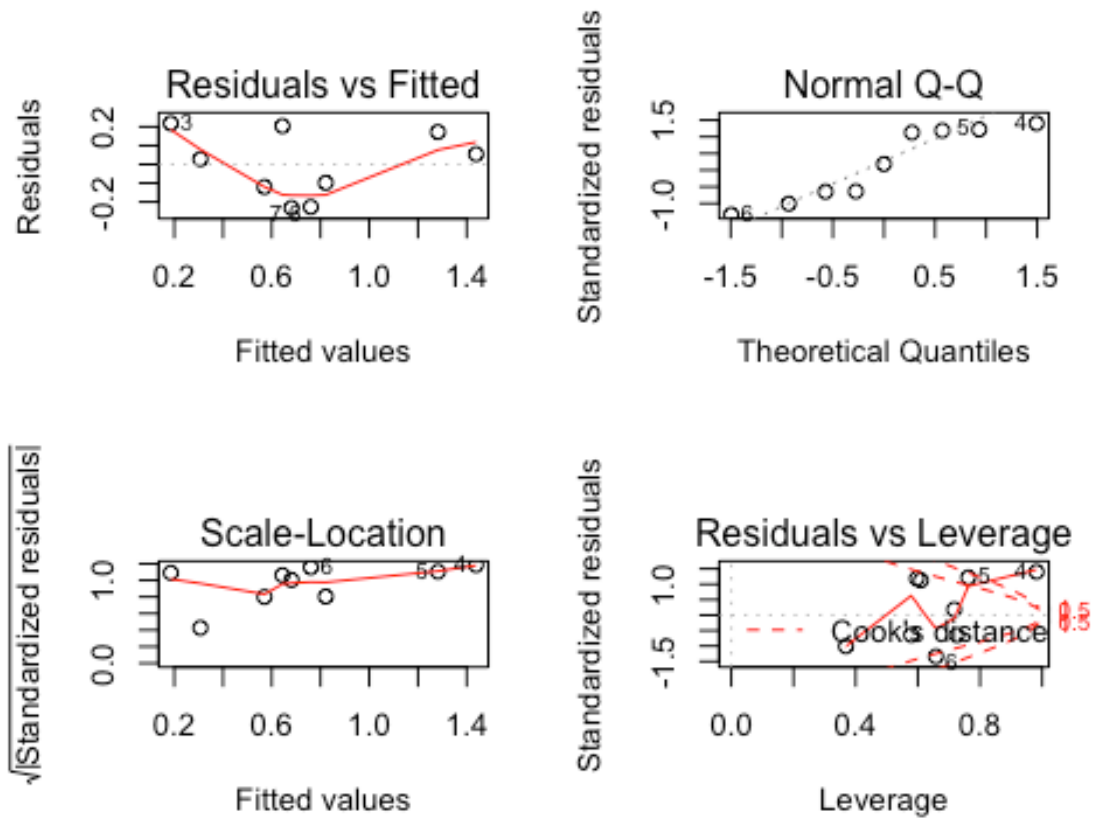
```
wood_dkc3 <- wood %>% filter(Decay_class == 3)

mod3.1 <- lm(Average_CO2 ~ Tree_species + Total_carbon_g_per_kg + Total_Phosp
horus_g_per_kg + lignin_pct, data=wood_dkc3)
summary(mod3.1)

##
## Call:
## lm(formula = Average_CO2 ~ Tree_species + Total_carbon_g_per_kg +
##     Total_Phosphorus_g_per_kg + lignin_pct, data = wood_dkc3)
##
## Residuals:
##        1        2        3        4        5        6        7        8
## -0.12025 -0.09827  0.21852  0.05433  0.17236 -0.22669 -0.23209  0.20408
##        9
##  0.02801
##
## Coefficients:
##                              Estimate Std. Error t value Pr(>|t|)
```

```
## (Intercept)                 4.987860   3.305601   1.509    0.228
## Tree_speciesLX              0.231284   0.452830   0.511    0.645
## Tree_speciesSN              0.461017   0.389484   1.184    0.322
## Total_carbon_g_per_kg      -0.012712   0.008088  -1.572    0.214
## Total_Phosphorus_g_per_kg   1.978470   1.168513   1.693    0.189
## lignin_pct                  0.048483   0.044828   1.082    0.359
##
## Residual standard error: 0.29 on 3 degrees of freedom
## Multiple R-squared:   0.84,  Adjusted R-squared:  0.5734
## F-statistic: 3.151 on 5 and 3 DF,  p-value: 0.1869
```

```r
vif(mod3.1)
```

```
##                                GVIF Df GVIF^(1/(2*Df))
## Tree_species               10.084610  2        1.782029
## Total_carbon_g_per_kg       2.176807  1        1.475401
## Total_Phosphorus_g_per_kg   3.462959  1        1.860903
## lignin_pct                  4.046438  1        2.011576
```

```r
par(mfrow=c(2,2))
plot(mod3.1)
```




```r
par(mfrow=c(1,1))
```

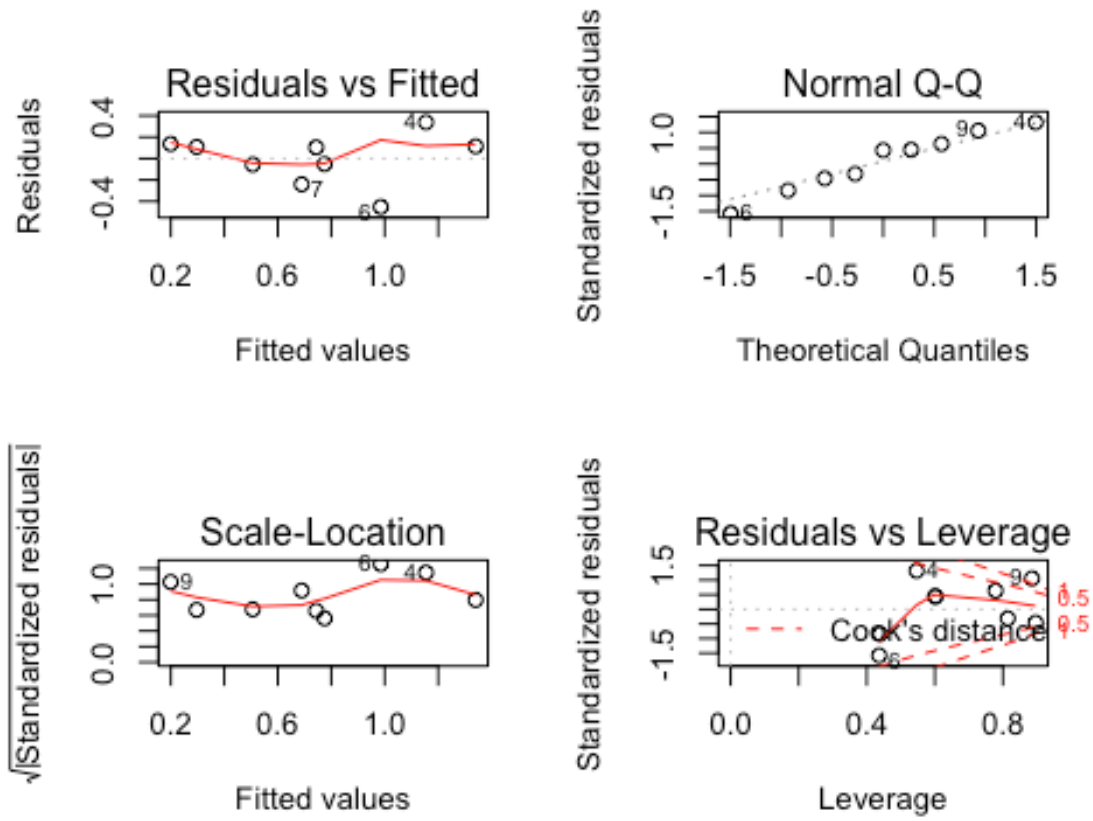
```r
mod3.1 <- lm(Average_CO2 ~ Tree_species + Total_carbon_g_per_kg + Total_Nitro
gen_g_per_kg + lignin_pct, data=wood_dkc3)
summary(mod3.1)
```

```
##
## Call:
## lm(formula = Average_CO2 ~ Tree_species + Total_carbon_g_per_kg +
##     Total_Nitrogen_g_per_kg + lignin_pct, data = wood_dkc3)
##
## Residuals:
##        1        2        3        4        5        6        7        8
## -0.05622 -0.05175  0.10797  0.33802  0.11382 -0.45185 -0.24040  0.10428
##        9
##  0.13612
##
## Coefficients:
##                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)              6.597447   4.439941   1.486    0.234
## Tree_speciesLX           0.140959   1.070537   0.132    0.904
## Tree_speciesSN           0.307366   0.552132   0.557    0.617
## Total_carbon_g_per_kg   -0.014093   0.010568  -1.333    0.275
## Total_Nitrogen_g_per_kg  0.164312   0.262019   0.627    0.575
## lignin_pct              -0.006041   0.125806  -0.048    0.965
##
## Residual standard error: 0.3813 on 3 degrees of freedom
## Multiple R-squared:  0.7234, Adjusted R-squared:  0.2625
## F-statistic:  1.57 on 5 and 3 DF,  p-value: 0.3772
```

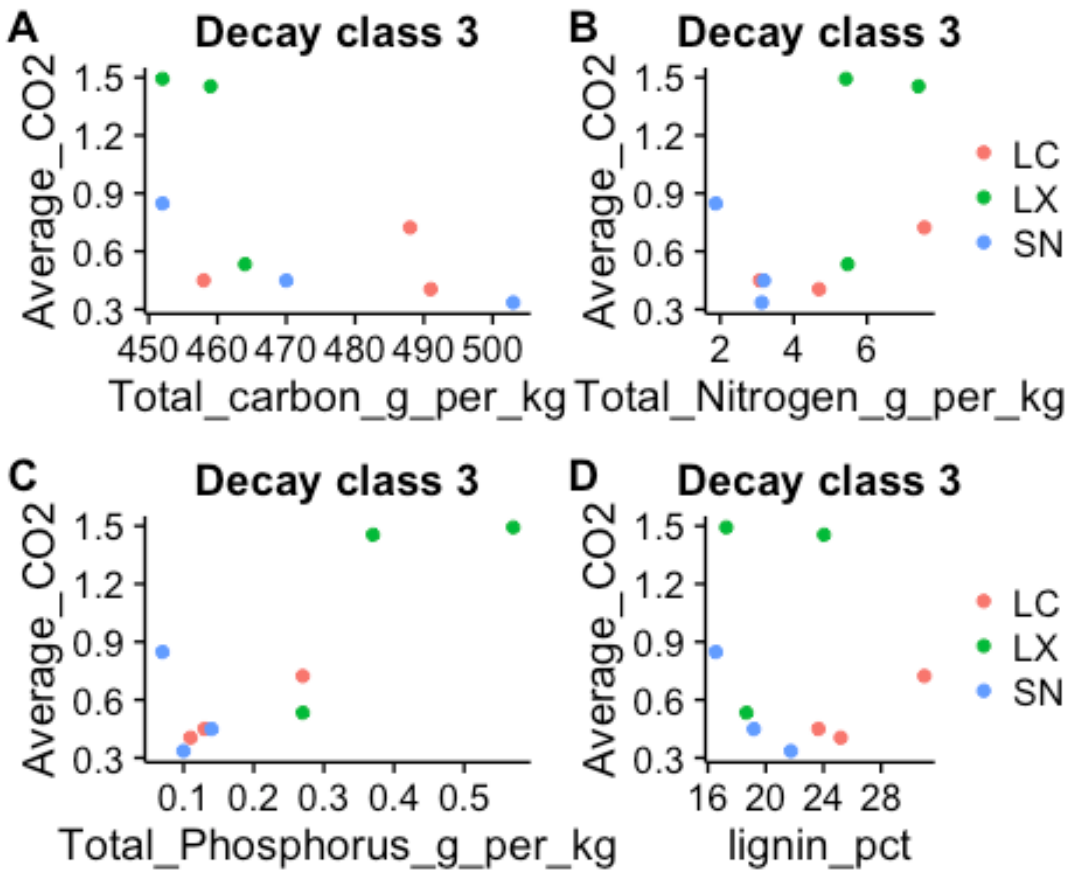
```r
vif(mod3.1)
```

```
##                              GVIF Df GVIF^(1/(2*Df))
## Tree_species            31.380994  2        2.366828
## Total_carbon_g_per_kg    2.149593  1        1.466149
## Total_Nitrogen_g_per_kg 15.056027  1        3.880210
## lignin_pct              18.432599  1        4.293320
```

```r
par(mfrow=c(2,2))
plot(mod3.1)
```




```
par(mfrow=c(1,1))
```




```
sessionInfo()

## R version 3.3.0 (2016-05-03)
## Platform: x86_64-apple-darwin13.4.0 (64-bit)
## Running under: OS X 10.11.5 (El Capitan)
##
## locale:
## [1] en_GB.UTF-8/en_GB.UTF-8/en_GB.UTF-8/C/en_GB.UTF-8/en_GB.UTF-8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] car_2.1-2      corrplot_0.77  cowplot_0.6.2  ggplot2_2.1.0
## [5] dplyr_0.4.3    xlsx_0.5.7     xlsxjars_0.6.1 rJava_0.9-8
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_0.12.5        formatR_1.4        nloptr_1.0.4
##  [4] plyr_1.8.4         tools_3.3.0        digest_0.6.9
##  [7] lme4_1.1-12        evaluate_0.9       gtable_0.2.0
```

```
## [10] nlme_3.1-128       lattice_0.20-33    mgcv_1.8-12
## [13] Matrix_1.2-6       DBI_0.4-1          yaml_2.1.13
## [16] parallel_3.3.0     SparseM_1.7        stringr_1.0.0
## [19] knitr_1.13         MatrixModels_0.4-1 grid_3.3.0
## [22] nnet_7.3-12        R6_2.1.2           rmarkdown_0.9.6
## [25] minqa_1.2.4        magrittr_1.5       scales_0.4.0
## [28] htmltools_0.3.5    MASS_7.3-45        splines_3.3.0
## [31] assertthat_0.1     pbkrtest_0.4-6     colorspace_1.2-6
## [34] labeling_0.3       quantreg_5.26      stringi_1.1.1
## [37] lazyeval_0.1.10    munsell_0.4.3
```

# Higher fungal diversity is correlated with lower $CO_2$ emissions from dead wood in a natural forest:

## <u>S3 Sandau et al. analyses</u>

# fungus_Sandau_analysis.R

## *Negorashi2011*

## *Thu Oct 30 17:34:54 2014*

```
##############################################
### R routine to include correlation structure in a linear model.
### (modified from Sandau et al 2014 in MEE by Viorel D. Popescu)
##############################################
## MAIN FUNCTIONS
##############################################
# FOR ALL FUNCTIONS THE INPUT PARAMETERS ARE:
# y = RESPONSE VARIABLE
# x = DESIGN MATRIX OF EXPLANATORY VARIABLES
# zf = DESIGN MATRIX FOR THE FIRST RANDOM EFFECT
# zt = DESIGN MATRIX FOR THE SECOND RANDOM EFFECT
# M = CORRELATION MATRIX

# THE OUTPUT IS A LIST CONTAINING:
# beta = MAXIMUM LIKELIHOOD ( OR RESTRICTED MAXIMUM LIKELIHOOD ) ESTIMATION OF THE FIXED
PARAMETERS
# se = STANDARD ERRORS OF THE FIXED PARAMETERS
# T = T-VALUES OF THE FIXED PARAMETERS
# pvalue = P-VALUE OF THE FIXED PARAMETERS
# lambda = LAMBDA PARAMETER FOR THE STRENGTH OF THE CORRELATION STRUCTURE
# sigma2 = OVERALL VARIANCE OF THE RESIDUALS
# loglike = MAXIMUM LOG-LIKELIHOOD ESTIMATION ( OR RESTRICTED LOG-LIKELIHOOD )



rm(list=ls())

# Run these functions once before all the Sandau et al. analyses #
####  START FUNCTIONS for MODEL W/CORRELATION STRUCTURE m_corr MODEL ##################
###############
#MAXIMUM LIKELIHOOD ESTIMATOR WITH CORRELATION STRUCTURE

fit_ML <- function(y,X,M){

  n <- length(y)
  p <- dim(X)[2]
  f <- function(a){-profile_ML_psi_lambda(1/(1+exp(-a[1])),y,X,M)}
  out <- optim(c(0), f, method="SANN")
  print(out)
  lambda <- 1 / (1 + exp(-out$par[1]))
  M2 <- M * lambda + diag(diag(M)) * (1-lambda)
  Pi <- solve(M2)
  out2 <- ML_beta_sigma(y,X,Pi)
```

```
  T <- out2$beta / out2$se
  pvalue <- dt(T,n-p)
  loglike <- -0.5 * n * log(2*pi) -1/(2*out2$sigma2) * t(y - X %*% out2$beta) %*% Pi  %*
% (y - X %*% out2$beta) -0.5 * n * log(out2$sigma2) + 0.5 * determinant(Pi,logarithm=TRU
E)$modulus[1]
  out3 <- c(out2, list(T = T, pvalue = pvalue, lambda = lambda, loglike = as.vector(logl
ike)))
  out3

}

## profile function

ML_beta_sigma <- function(y,X,Pi){

  n <- dim(X)[1]
  p <- dim(X)[2]
  beta <- solve( (t(X) %*% Pi %*% X), t(X) %*% Pi %*% y)
  sigma2 <- 1/n * t(y - X %*% beta) %*% Pi  %*% (y - X %*% beta)
  se <- sqrt( diag( solve(t(X) %*% Pi %*% X) ) * sigma2 )
  out <- list(beta = as.vector(beta), sigma2 = sigma2, se = se)
  out

}

###

profile_ML_psi_lambda <- function(lambda,y,X,M){

  n <- length(y)
  M2 <- M * lambda + diag(diag(M)) * (1-lambda)
  Pi <- solve(M2)
  out <- ML_beta_sigma(y,X,Pi)
  l <- -0.5 * n * log(out$sigma2) + 0.5 * determinant(Pi,logarithm=TRUE)$modulus[1]
  l

}
### END FUNCTIONS for MODEL W/CORRELATION STRUCTURE m_corr MODEL ####

### START FUNCTIONS for MODEL W/O CORRELATION STRUCTURE m_ref MODEL #####

fit_ML_without_lambda <- function(y,X){

  M <- diag(rep(1,length(y)))
  n <- length(y)
  p <- dim(X)[2]
  f <- function(a){-profile_ML_psi(y,X,M)}
  #out <- optim(c(0,0), f)
```

```
  Pi <- solve(M)
  out2 <- ML_beta_sigma(y,X,Pi)
  T <- out2$beta / out2$se
  pvalue <- dt(T,n-p)
  loglike <- -0.5 * n * log(2*pi) -1/(2*out2$sigma2) * t(y - X %*% out2$beta) %*% Pi  %*
% (y - X %*% out2$beta) -0.5 * n * log(out2$sigma2) + 0.5 * determinant(Pi,logarithm=TRU
E)$modulus[1]
  out3 <- c(out2, list(T = T, pvalue = pvalue, loglike = as.vector(loglike)))
  out3

}

ML_beta_sigma <- function(y,X,Pi){

  n <- dim(X)[1]
  p <- dim(X)[2]
  beta <- solve( (t(X) %*% Pi %*% X), t(X) %*% Pi %*% y)
  sigma2 <- 1/n * t(y - X %*% beta) %*% Pi  %*% (y - X %*% beta)
  se <- sqrt( diag( solve(t(X) %*% Pi %*% X) ) * sigma2 )
  out <- list(beta = as.vector(beta), sigma2 = sigma2, se = se)
  out

}

profile_ML_psi <- function(y,X,M){
  n <- length(y)
  Pi <- solve(M)
  out <- ML_beta_sigma(y,X,Pi)
  l <- -0.5 * n * log(out$sigma2) + 0.5 * determinant(Pi,logarithm=TRUE)$modulus[1]
  l
}
#### END FUNCTIONS for MODEL W/O CORRELATION STRUCTURE m_ref MODEL ####


#### START THE ANALYSES ##########################################################
library(vegan)
```

```
## Loading required package: permute
## Loading required package: lattice
## This is vegan 2.0-10
```

```r
library(car)

setwd("~/Dropbox/Working Docs/Barcode soup censuses/Schaefer, Fungus/analyses 31 Oct 201
4 Doug Viorel")

# June2012CO2.crop.lc.txt #
# June2012CO2.crop.lx.txt #
# June2012CO2.crop.sn.txt, omitting influential woodpieceID=110 #
# June2013CO2.crop.lc.txt #
# June2013CO2.crop.lx.txt, omitting woodpieceID=95 because no CO2 value #
# June2013CO2.crop.sn.txt, omitting woodpieceID=31 because no CO2 value#
# June2012CO2.uclust.lc.txt #
# June2012CO2.uclust.lx.txt #
# June2012CO2.uclust.sn.txt, omitting influential woodpieceID=110 #
# June2013CO2.uclust.lc.txt #
# June2013CO2.uclust.lx.txt, omitting woodpieceID=95 because no CO2 value #
# June2013CO2.uclust.sn.txt, omitting woodpieceID=31 because no CO2 value#

filename <- 'June2012CO2.crop.lc.txt' # i paste each file name from the list above into
this command, select all (which starts the run by clearing the workspace), and run all c
ommands. Then repeat for each file
fung <- read.table(filename,header=TRUE)
colnames(fung)[2] <- "CO2"
names(fung[,1:2])
```

```
## [1] "Fungus.apn.lc.run1.sep.env.woodpieceID"
## [2] "CO2"
```

```r
# str(fung)
nrow(fung) # because number of rows differs across months
```

```
## [1] 25
```

```r
ncol(fung) # because number of cols differs between CROP and uclust
```

```
## [1] 2866
```

```r
# calculate the similarity matrix (1 - Jaccard dissimilarity)
# rows and cols in the 'fung' matrix vary across dates and wood species
M.fung.dis <- as.dist(vegdist(fung[1:nrow(fung),3:ncol(fung)], binary=T, method="jaccard
"))
M.fung <- 1-as.matrix(M.fung.dis)

# Predicted variable is CO2 emissions
Y.fung.CO2 = as.matrix(fung$CO2)

# Predictor variable is the Shannon diversity index
# rows and cols in the 'fung' matrix vary across dates and wood species
X.fung.div = as.matrix(diversity(fung[1:nrow(fung),3:ncol(fung)]))

# Get the design matrix for the model [Y.fung.CO2 ~ X.fung.div]
X.fungi = as.matrix(model.matrix(Y.fung.CO2~X.fung.div))



## Fit LM MODEL ##

m_lm=lm(Y.fung.CO2~X.fung.div)
summary(m_lm)
```

```
##
## Call:
## lm(formula = Y.fung.CO2 ~ X.fung.div)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -1.2371 -0.3129 -0.1149  0.2254  1.9306
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)   2.4957     0.4281   5.830 6.11e-06 ***
## X.fung.div   -0.5366     0.1814  -2.957  0.00706 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.7508 on 23 degrees of freedom
## Multiple R-squared:  0.2755, Adjusted R-squared:  0.244
## F-statistic: 8.746 on 1 and 23 DF,  p-value: 0.007061
```

```
plot(X.fung.div, Y.fung.CO2)
abline(lm(Y.fung.CO2 ~ X.fung.div))



#### Fit Model WITH CORRELATION STRUCTURE


m_corr <- fit_ML(Y.fung.CO2,X.fungi,M.fung)
```

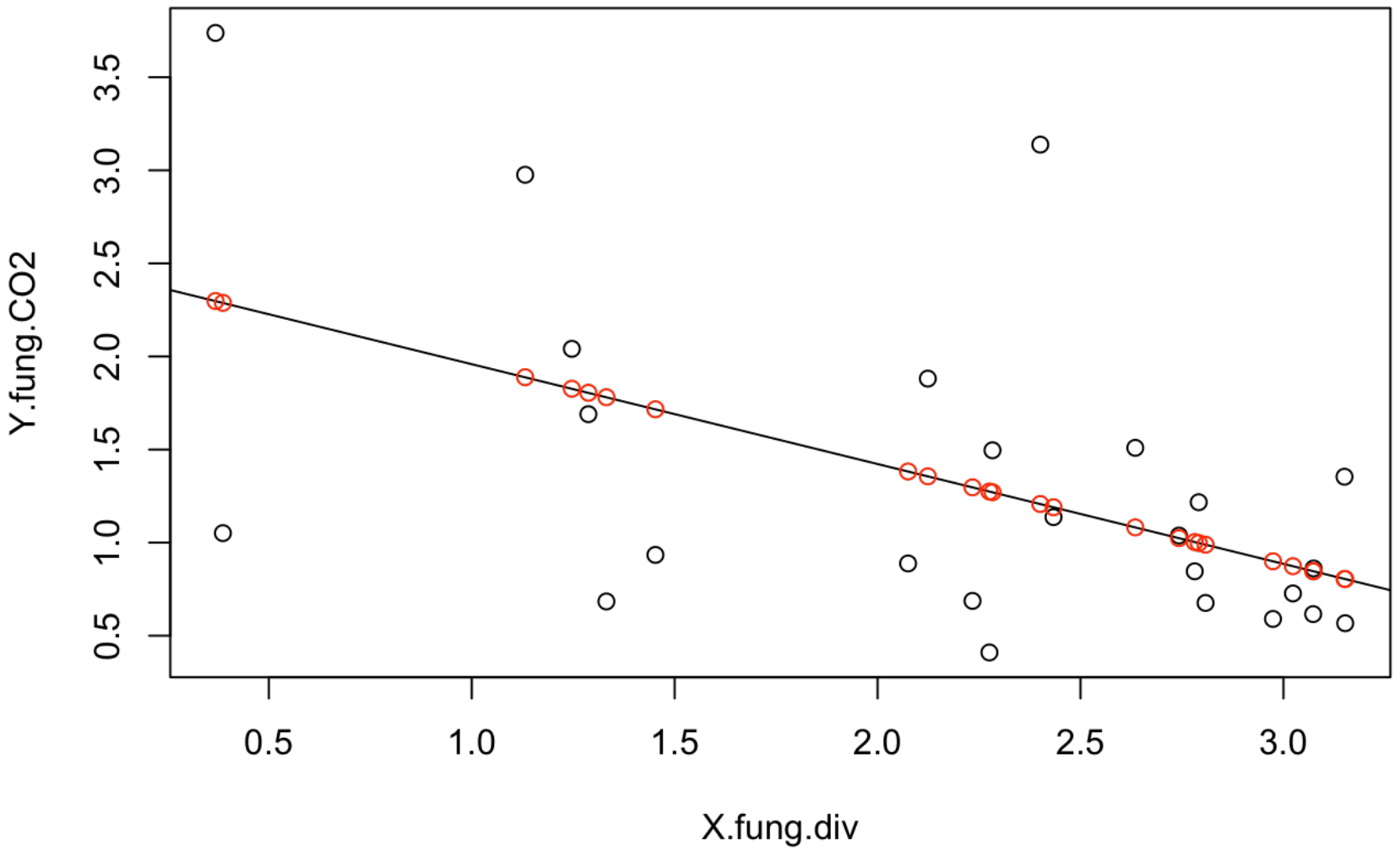
```
## $par
## [1] -17.52438
##
## $value
## [1] -8.209146
##
## $counts
## function gradient
##    10000       NA
##
## $convergence
## [1] 0
##
## $message
## NULL
```

```
m_corr # look at the lambda value, which is the strength of the correlation structure. W
ith input dataset JuneOTU.lc.txt, the lambda value is close to zero, meaning almost no e
ffect of the community structure on the amount of CO2 released.  Consequently, the coeff
icient estimates, p-values and loglikelihood are similar to the lm model.
```

```
## $beta
## [1]  2.4957317 -0.5366139
##
## $sigma2
##           [,1]
## [1,] 0.5185433
##
## $se
## [1] 0.4106264 0.1740396
##
## $T
## [1]  6.077865 -3.083286
##
## $pvalue
## [1] 4.020548e-06 6.212384e-03
##
## $lambda
## [1] 2.450513e-08
##
## $loglike
## [1] -27.26432
```

```
predicted = m_corr$beta[1] + m_corr$beta[2]*X.fung.div
predicted; points(X.fung.div, predicted, col="red") # With the JuneOTU.lc.txt dataset, t
he
```

```
##         [,1]
## 1  0.8051847
## 2  0.8733322
## 3  2.2978963
## 4  1.2073624
## 5  0.8997118
## 6  0.8459969
## 7  1.8267251
## 8  1.2746412
## 9  1.7810741
## 10 1.0817882
## 11 1.7162385
## 12 1.3560805
## 13 0.9979075
## 14 2.2881198
## 15 1.8884770
## 16 1.3821675
## 17 1.0240120
## 18 0.9888807
## 19 1.0031981
## 20 0.8466022
## 21 1.2706007
## 22 0.8042258
## 23 1.1899057
## 24 1.2969998
## 25 1.8048714
```




```
### END MODEL WITH CORRELATION STRUCTURE



### FIT REFERENCE MODEL (no correlation structure)
m_ref <- fit_ML_without_lambda(Y.fung.CO2,X.fungi)
m_ref
```

```
## $beta
## [1]  2.4957317 -0.5366139
##
## $sigma2
##           [,1]
## [1,] 0.5185433
##
## $se
## [1] 0.4106264 0.1740396
##
## $T
## [1]  6.077865 -3.083286
##
## $pvalue
## [1] 4.020548e-06 6.212384e-03
##
## $loglike
## [1] -27.26432
```

```
### END REFERENCE MODEL



# estimation of p-value of lambda (see Zuur et al. 2009, pp. 123-124)
pl <- 0.5*(1-pchisq(m_corr$loglike-m_ref$loglike,1))
pl
```

```
## [1] 0.5
```

```
# write results to a table
results <- cbind(filename,m_corr$lambda,pl)
write.table(results,"Sandau et al results.txt",append = TRUE,sep="\t",col.names=FALSE,qu
ote=FALSE)
```

# Higher fungal diversity is correlated with lower $CO_2$ emissions from dead wood in a natural forest:

## S4 Conventional Community Analyses

**Supporting Information S4**. **Conventional community analyses to test for an effect of fungal community composition *per se* on $CO_2$ emissions.**

**Methods**

We removed singleton OTUs (OTUs occurring in only one sample) and visualized the OTU tables using correspondence analysis (*cca()* in *R*). We tested the hypothesis that $CO_2$ emissions explain variation in community composition by using: (1) *cca*(OTUtable ~ $CO_2$) + *anova*(by="term", perm=9999) in *vegan* (***Oksanen et al., 2013***) and (2) *manyglm*(OTUtable ~ $CO_2$, family="negative binomial") + *anova*(resamp="pit.trap", nBoot=999) in *mvabund* (***Warton et al., 2012***). As four versions of each OTU table were tested, we Bonferroni-corrected for multiple tests by multiplying the resulting p-values by 4. *mvabund* is a multivariate implementation of generalized linear models, and unlike dissimilarity-based methods such as *cca*, *mvabund* does not confound location with dispersion effects, which can inflate both type 1 and 2 errors (***Warton et al., 2012***). Based on the correspondence-analysis plots, we deemed some low-diversity wood pieces to be possible influential outliers (*i.e.* individual samples likely to be largely responsible for any significant community effects), and we removed these data points and repeated the analyses.

**Results**

After Bonferroni correction, no June 2012 samples showed significant effects of fungal composition on CO2 emissions, regardless of wood species, statistic, OTU-clustering method, or rarefaction (Table S4).

For June 2013 samples, CCA detected marginally significant effects of composition for all three wood species after Bonferroni correction, but for LC and LX, statistical significance relied on one or two highly dissimilar, low-fungal-diversity wood samples (Main text, Fig. 2). For SN, significant CCA effects were robust to the removal of three potentially influential points, but the *mvabund* test for SN was non-significant after Bonferroni correction. Overall, our data do not reject the null hypothesis that higher fungal species diversity per se, and not any particular fungal species, is responsible for reducing CO2 emissions.

**References**

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin RB, O'Hara RB, et al. 2013. vegan: Community Ecology R package version 2.0-10. Available at: http://CRAN.R-project.org/package=vegan. Last accessed 30 May 2014.

Warton DI, Wright ST, Wang Y. 2012. Distance-based multivariate analyses confound location and dispersion effects. *Meth Ecol Evol* **3**:89-101. doi:10.1111/j.2041-210X.2011.00127.x.

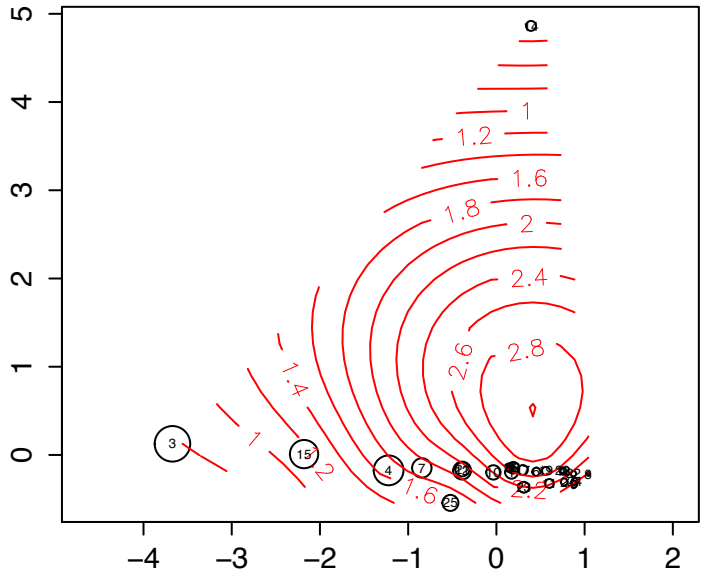
**Table S4**. Inferring a 'pure diversity' effect of fungal communities on $CO_2$ emissions. For each of the six fungal communities visualized in Figure 2, two statistical methods (canonical correspondence analysis and *mvabund*, see Methods), were used to test for significant effects of fungal community composition on $CO_2$ emissions. For all June 2012 $CO_2$ samples, no significant effects of fungal community (September 2012) were found. For the June 2013 $CO_2$ samples, marginally significant effects after Bonferroni correction ($p < 0.05$) were detected, but only by CCA, not by *mvabund*. Moreover, the significant CCA effects in the two *Lithocarpus* species were not robust to the removal of a few outlying, low-diversity wood pieces, as seen in Fig. 2. Wood species are indicated by LC, LX, SN, for *Lithocarpus chintungensis*, *L. xylocarpus*, and *Schima noronhae*, respectively.

| $CO_2$ sample date[1] | Species | Test method | Low-diversity wood pieces removed?[2] | p-value range[3] |
|---|---|---|---|---|
| June 2012 | LC | CCA | no, full dataset | 0.10-0.37 |
| | | | 14 removed | 0.13-0.39 |
| | | *mvabund* | no, full dataset | 0.15-0.23 |
| | LX | CCA | no, full dataset | 0.04-0.09 |
| | | | 9, 15 removed | 0.17-0.26 |
| | | *mvabund* | no, full dataset | 0.39-0.47 |
| | SN | CCA | no, full dataset | 0.09-0.15 |
| | | | 7, 10 removed | 0.04-0.24 |
| | | *mvabund* | no, full dataset | 0.06-0.54 |
| June 2013 | LC | CCA | no, full dataset | 0.01-0.02* |
| | | | 9, 17 removed | 0.17-0.46 |
| | | *mvabund* | no, full dataset | 0.03-0.08 |
| | LX | CCA | no, full dataset | 0.01-0.03* |
| | | | 9 removed | 0.04-0.18 |
| | | *mvabund* | no, full dataset | 0.15-0.32 |
| | SN | CCA | no, full dataset | 0.01-0.02* |
| | | | 10, 11, 14 removed | 0.01-0.05* |
| | | *mvabund* | no, full dataset | 0.03-0.05 |

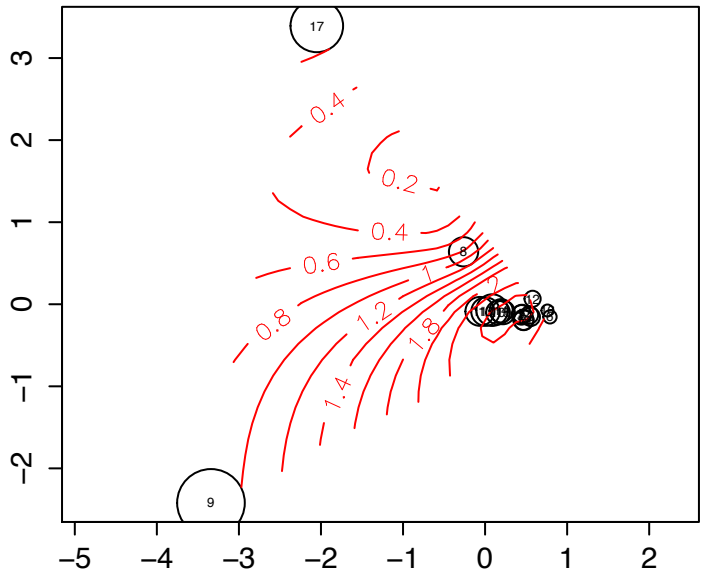
[1] September 2012 fungal community was tested against June 2012 $CO_2$ emissions, as in Fig. 1.
[2] Numbers refer to points in Fig. 2.
[3] Asterisks indicate formally significant effects ($p<0.05$) after Bonferroni correction for four tests: CROP/non-rarefied, CROP/rarefied, *uclust*/non-rarefied, and *uclust*/rarefied. Original p-values reported here.

# S4 A. CROP OTU-picking, rarefied

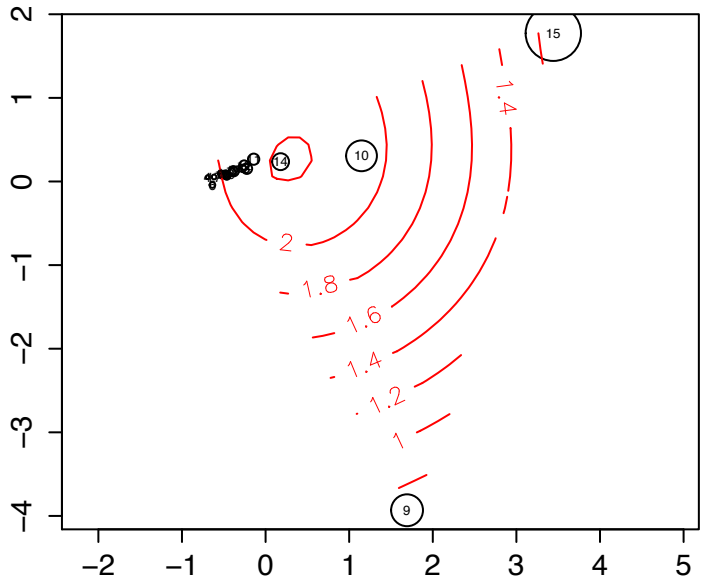
LC, June 2012 $CO_2$ ~ Sep 2012 Shannon



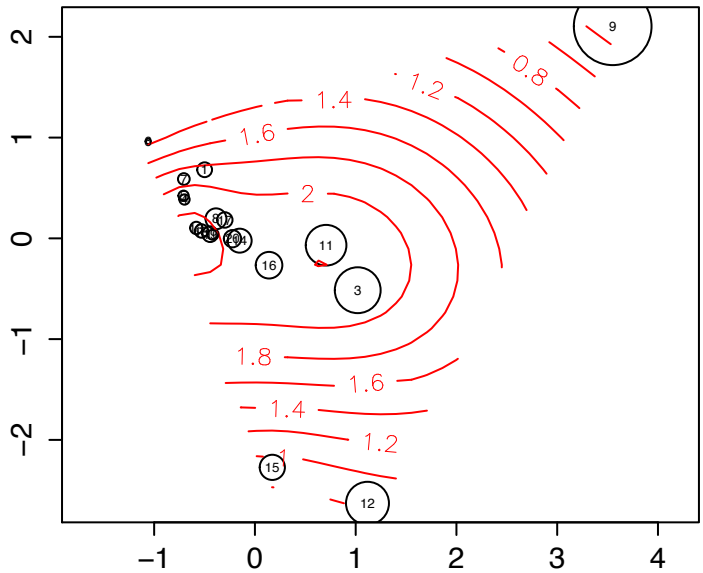
LC, June 2013 $CO_2$ ~ June 2013 Shannon



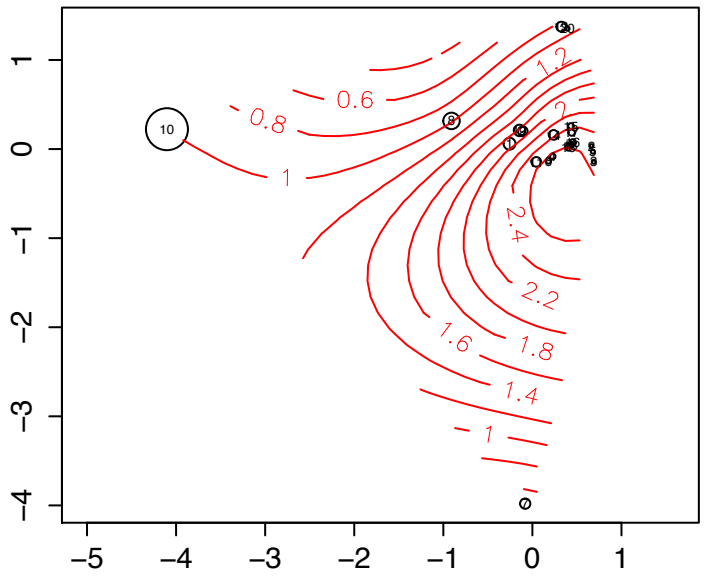
LX, June 2012 $CO_2$ ~ Sept 2012 Shannon



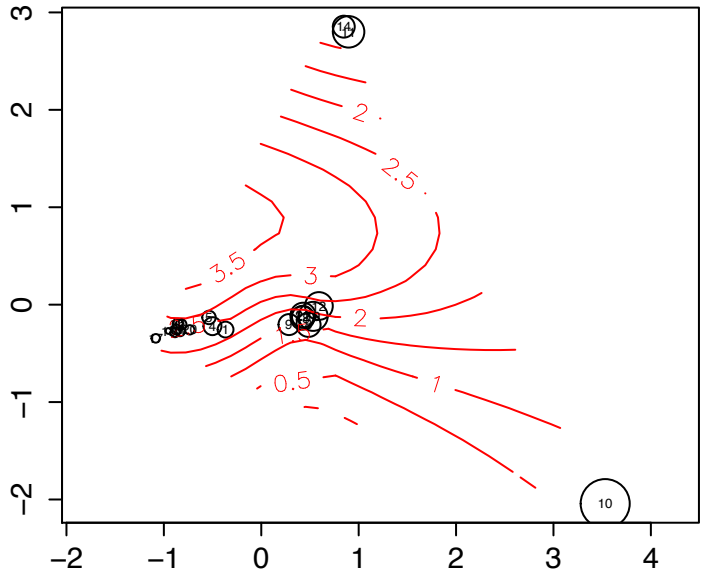
LX, June 2013 $CO_2$ ~ June 2013 Shannon



SN, June 2012 $CO_2$ ~ Sept 2012 Shannon

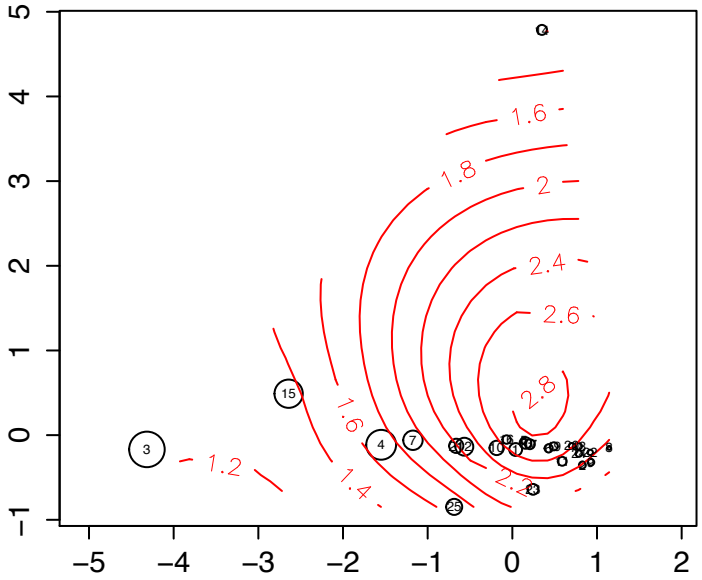


SN, June 2013 $CO_2$ ~ June 2013 Shannon

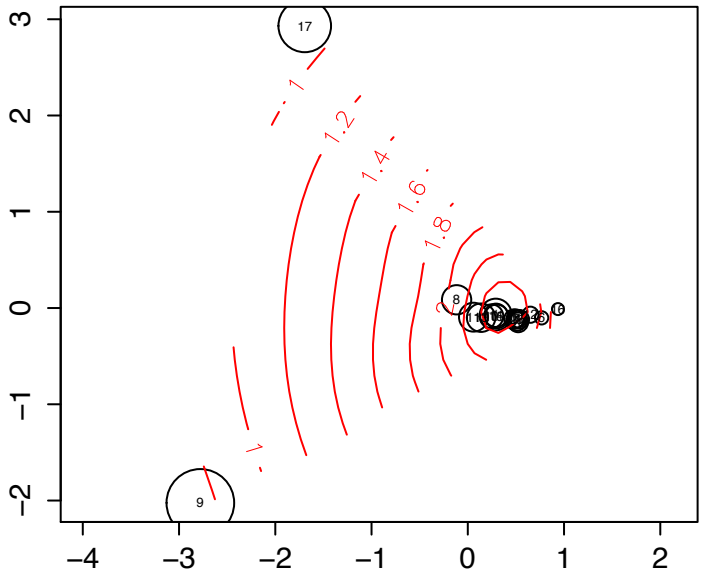


# S4 B. *uclust* OTU-picking, non-rarefied

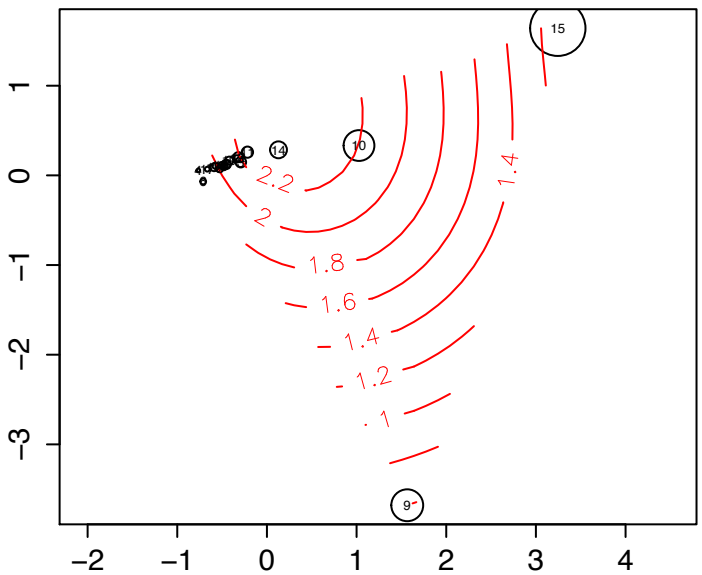
LC, June 2012 $CO_2$ ~ Sep 2012 Shannon



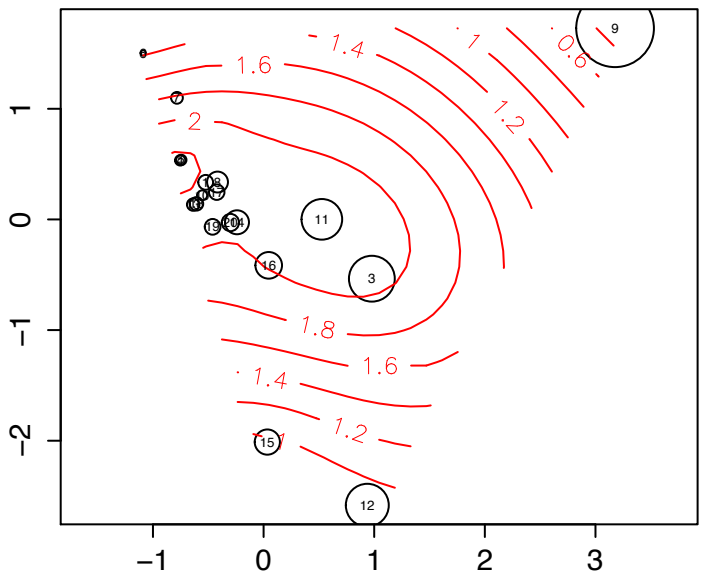
LC, June 2013 $CO_2$ ~ June 2013 Shannon



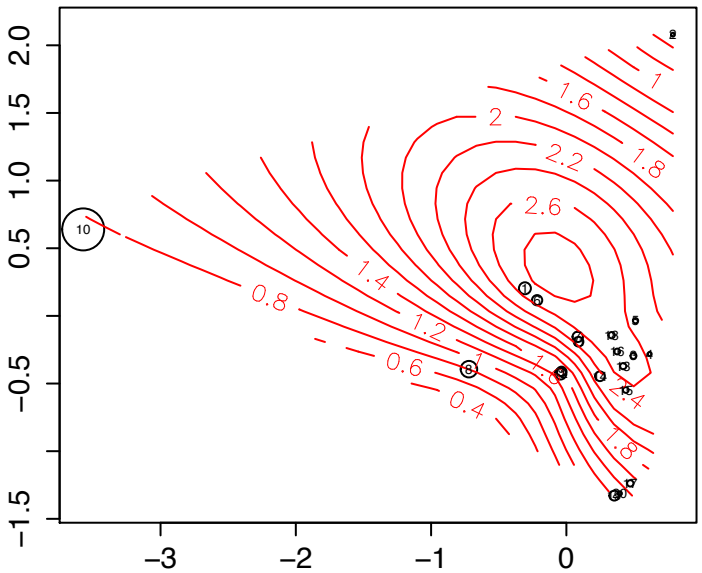
LX, June 2012 $CO_2$ ~ Sept 2012 Shannon



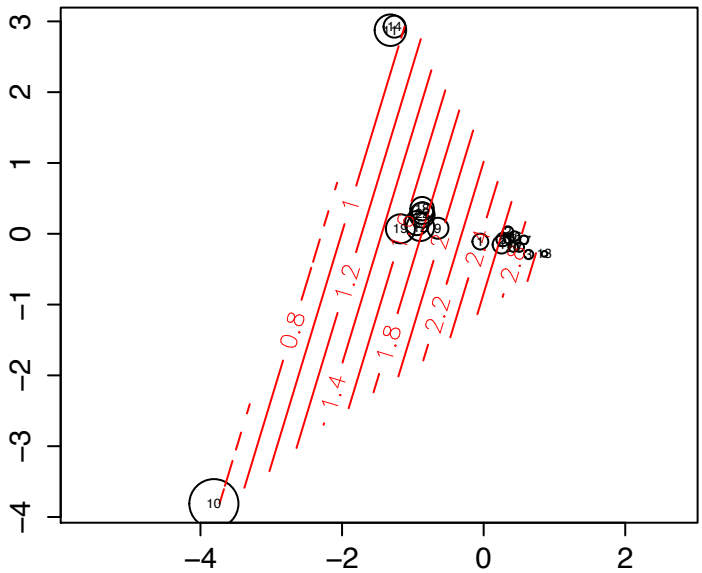
LX, June 2013 $CO_2$ ~ June 2013 Shannon



SN, June 2012 $CO_2$ ~ Sept 2012 Shannon

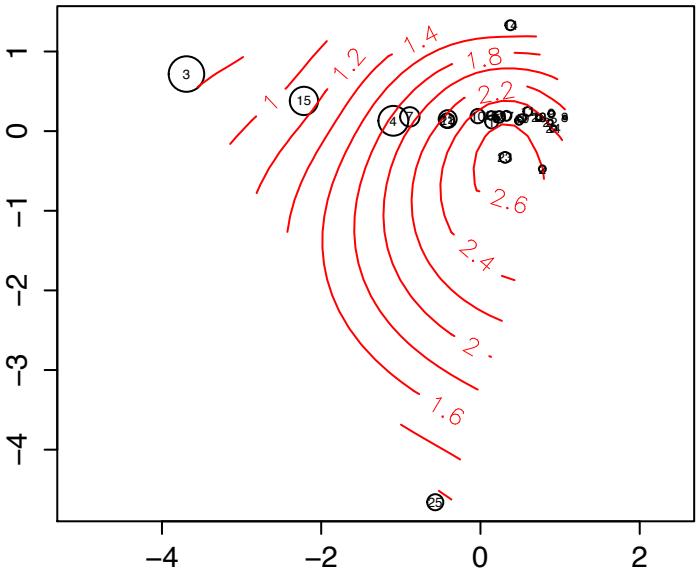


SN, June 2013 $CO_2$ ~ June 2013 Shannon

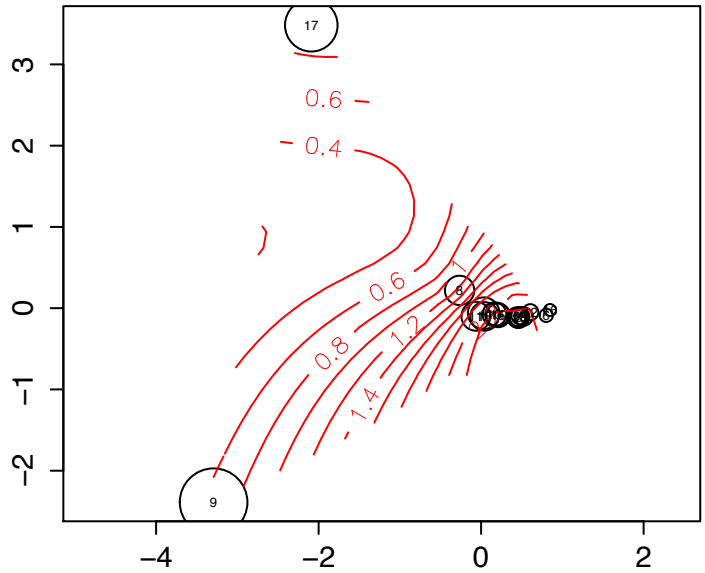


# S4 C. *uclust* OTU-picking, rarefied

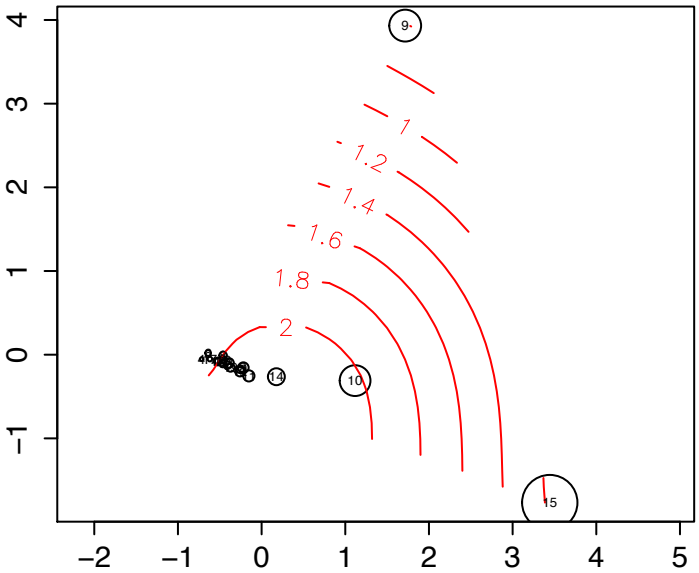
LC, June 2012 $CO_2$ ~ Sep 2012 Shannon



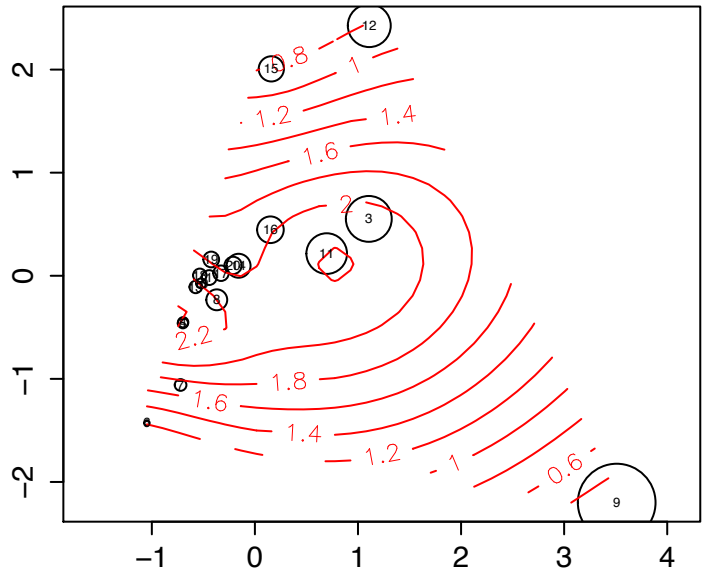

LC, June 2013 $CO_2$ ~ June 2013 Shannon



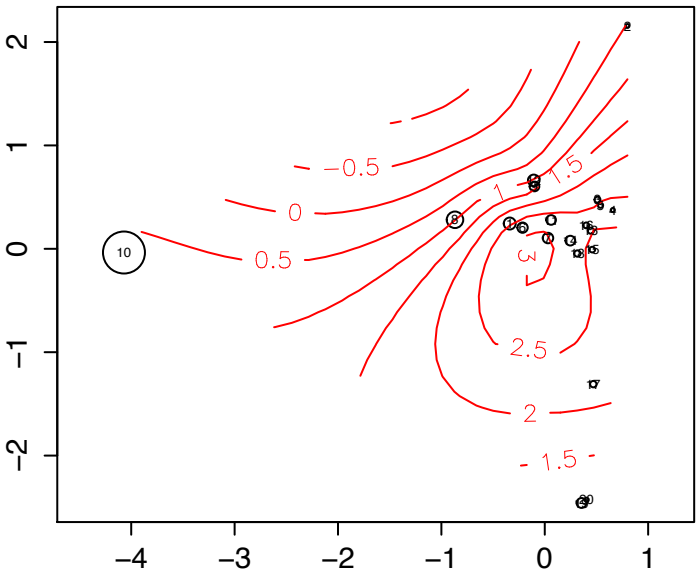

LX, June 2012 $CO_2$ ~ Sept 2012 Shannon



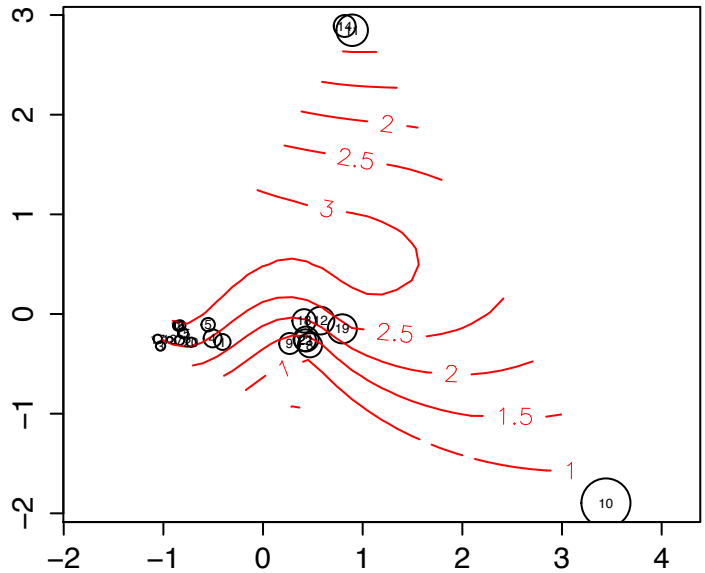

LX, June 2013 $CO_2$ ~ June 2013 Shannon




SN, June 2012 $CO_2$ ~ Sept 2012 Shannon



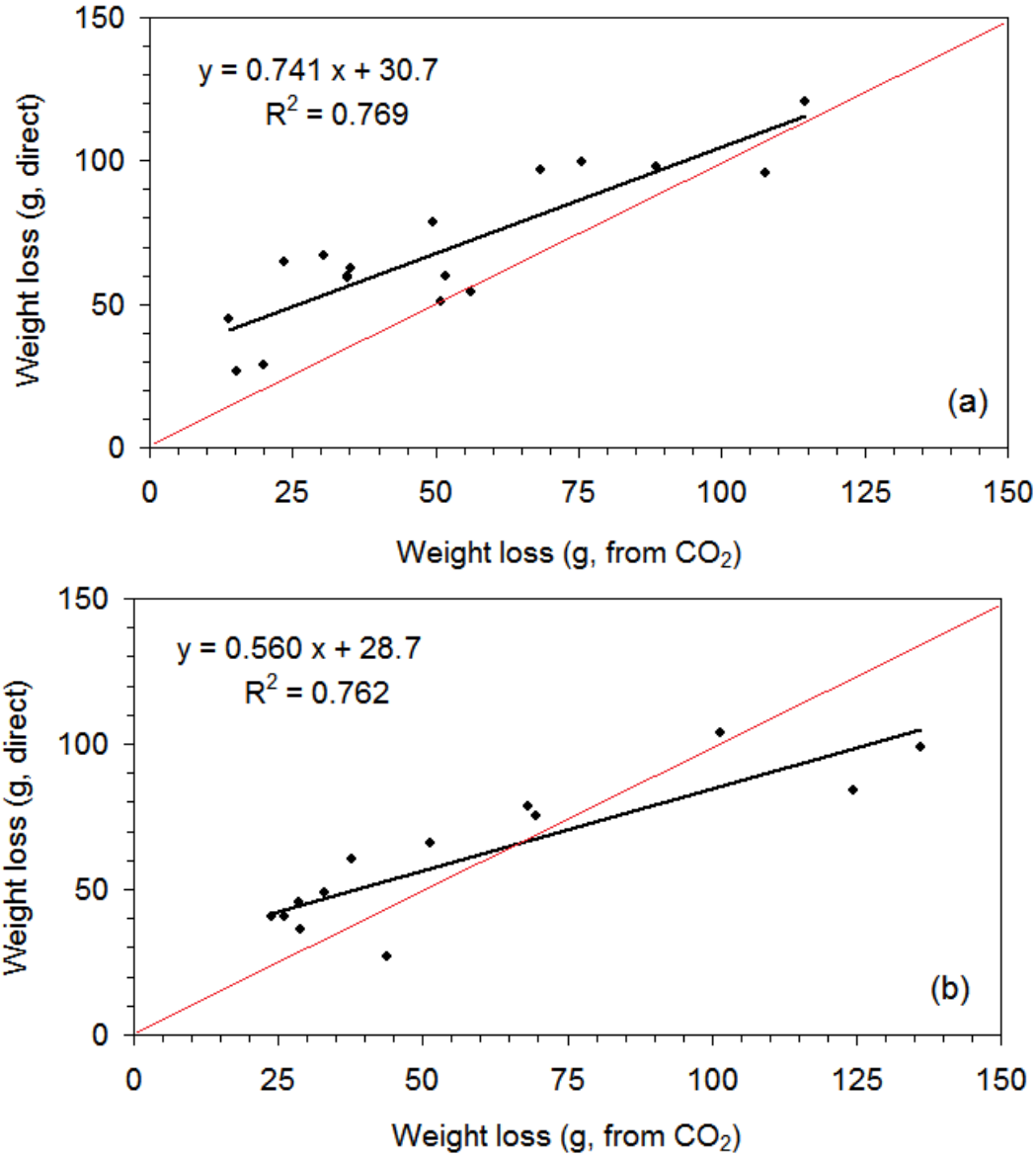

SN, June 2013 $CO_2$ ~ June 2013 Shannon

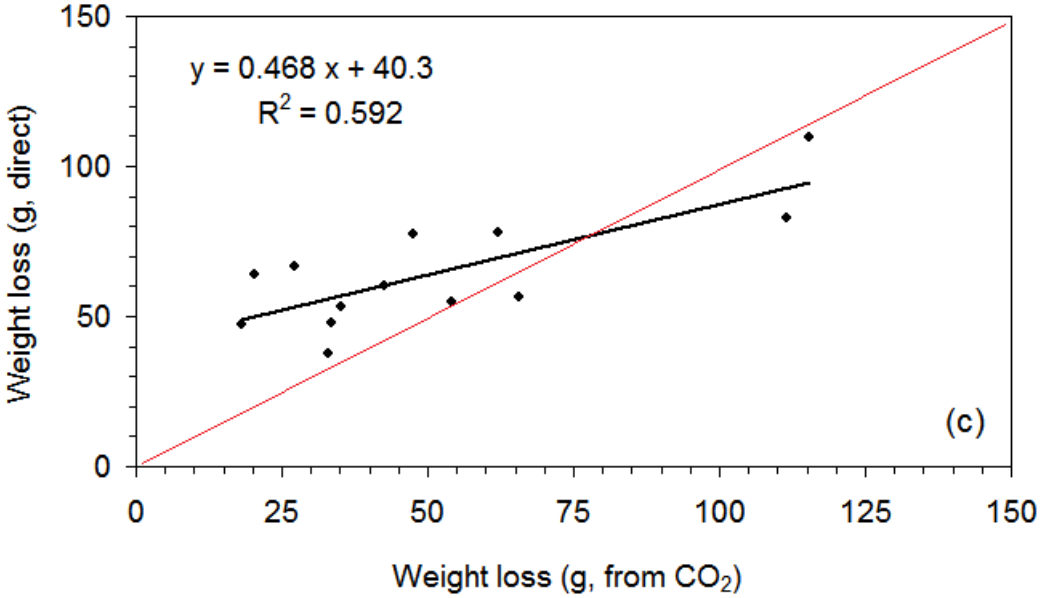


# Higher fungal diversity is correlated with lower $CO_2$ emissions from dead wood in a natural forest:

## S5 Gravimetric vs $CO_2$ estimated loss

**Supporting Information S5.** Mass loss from wood pieces (g over 3 years) as measured gravimetrically and as estimated from average $CO_2$ emission rates in decay class 1 (DKC1, top), decay class 2 (middle), and decay class 3 (bottom). Black lines are linear regressions and red lines represent 1:1 correspondences.




y = 0.468 x + 40.3
R2 = 0.592
(c)
Weight loss (g, direct)
Weight loss (g, from CO2)
0
50
100
150
0
25
50
75
100
125
150

# Higher fungal diversity is correlated with lower $CO_2$ emissions from dead wood in a natural forest:

## <u>SI Bioinformatic Command Scripts</u>

```
  # Background Information.
  # 2012 samples (LC) were run in 3 regions in Jan 13, and 2 regions were
supplemented in Feb 13.
    2013 samples and 2012 samples (LX, SN) were run in 5 regions in Aug 13. 21
samples per region.
    On GSFLX454

  ############################################ split libraries
#####################################################
  #2012#
  validate_mapping_file.py -m FungusMap_1.txt -o mapping_output
  validate_mapping_file.py -m FungusMap_2.txt -o mapping_output
  validate_mapping_file.py -m FungusMap_3.txt -o mapping_output
  split_libraries.py -m FungusMap_1.txt -f 1.TCA.454Reads.fna -q
1.TCA.454Reads.qual -o split_library_output/1 -l 100 -L 500 -H 30 -z
truncate_only -b 10
  split_libraries.py -m FungusMap_2.txt -f 2.TCA.454Reads.fna -q
2.TCA.454Reads.qual -o split_library_output/2 -l 100 -L 500 -H 30 -z
truncate_only -b 10 -n 42576
  split_libraries.py -m FungusMap_3.txt -f 3.TCA.454Reads.fna -q
3.TCA.454Reads.qual -o split_library_output/3 -l 100 -L 500 -H 30 -z
truncate_only -b 10 -n 79479
  split_libraries.py -m FungusMap_1.txt -f 7.TCA.454Reads.fna -q
7.TCA.454Reads.qual -o split_library_output/7 -l 100 -L 500 -H 30 -z
truncate_only -b 10 -n 143667
  split_libraries.py -m FungusMap_2.txt -f 8.TCA.454Reads.fna -q
8.TCA.454Reads.qual -o split_library_output/8 -l 100 -L 500 -H 30 -z
truncate_only -b 10 -n 211778

  #2013#
  validate_mapping_file.py -m FungusMap_4.txt -o mapping_output
  validate_mapping_file.py -m FungusMap_5.txt -o mapping_output
  validate_mapping_file.py -m FungusMap_6.txt -o mapping_output
  validate_mapping_file.py -m FungusMap_7.txt -o mapping_output
  validate_mapping_file.py -m FungusMap_8.txt -o mapping_output
  split_libraries.py -m FungusMap_4.txt -f 4.TCA.454Reads.fna -q
4.TCA.454Reads.qual -o split_library_output/4 -l 100 -L 500 -H 30 -z
truncate_only -b 10
  split_libraries.py -m FungusMap_5.txt -f 5.TCA.454Reads.fna -q
5.TCA.454Reads.qual -o split_library_output/5 -l 100 -L 500 -H 30 -z
truncate_only -b 10 -n 86179
  split_libraries.py -m FungusMap_6.txt -f 6.TCA.454Reads.fna -q
6.TCA.454Reads.qual -o split_library_output/6 -l 100 -L 500 -H 30 -z
truncate_only -b 10 -n 167521
  split_libraries.py -m FungusMap_7.txt -f 7.TCA.454Reads.fna -q
7.TCA.454Reads.qual -o split_library_output/7 -l 100 -L 500 -H 30 -z
truncate_only -b 10 -n 256997
  split_libraries.py -m FungusMap_8.txt -f 8.TCA.454Reads.fna -q
8.TCA.454Reads.qual -o split_library_output/8 -l 100 -L 500 -H 30 -z
truncate_only -b 10 -n 351216


  ######################################Denoiser on QIIME pipeline
#################################################
  #2012#
  denoiser.py -i H2P9ZGK01.txt -f ../split_library_output/1/seqs.fna --primer
TCCTCCGCTTATTGATATGC -v -o denoise/1 -c -n 8
```

```
  denoiser.py -i H2P9ZGK02.txt -f ../split_library_output/2/seqs.fna --primer
TCCTCCGCTTATTGATATGC -v -o denoise/2 -c -n 8
  denoiser.py -i H2P9ZGK03.txt -f ../split_library_output/3/seqs.fna --primer
TCCTCCGCTTATTGATATGC -v -o denoise/3 -c -n 8
  denoiser.py -i H3RH8HN07.txt -f ../split_library_output/7/seqs.fna --primer
TCCTCCGCTTATTGATATGC -v -o denoise/7 -c -n 8
  denoiser.py -i H3RH8HN08.txt -f ../split_library_output/8/seqs.fna --primer
TCCTCCGCTTATTGATATGC -v -o denoise/8 -c -n 8

  inflate_denoiser_output.py -c denoise/1/centroids.fasta -s
denoise/1/singletons.fasta -f ../split_library_output/1/seqs.fna -d
denoise/1/denoiser_mapping.txt -o inflated_1_seqs.fna
  inflate_denoiser_output.py -c denoise/2/centroids.fasta -s
denoise/2/singletons.fasta -f ../split_library_output/2/seqs.fna -d
denoise/2/denoiser_mapping.txt -o inflated_2_seqs.fna
  inflate_denoiser_output.py -c denoise/3/centroids.fasta -s
denoise/3/singletons.fasta -f ../split_library_output/3/seqs.fna -d
denoise/3/denoiser_mapping.txt -o inflated_3_seqs.fna
  inflate_denoiser_output.py -c denoise/7/centroids.fasta -s
denoise/7/singletons.fasta -f ../split_library_output/7/seqs.fna -d
denoise/7/denoiser_mapping.txt -o inflated_7_seqs.fna
  inflate_denoiser_output.py -c denoise/8/centroids.fasta -s
denoise/8/singletons.fasta -f ../split_library_output/8/seqs.fna -d
denoise/8/denoiser_mapping.txt -o inflated_8_seqs.fna

  cat FungusMap_1.txt FungusMap_2.txt FungusMap_3.txt > Map_2012.txt
  cat inflated_1_seqs.fna inflated_2_seqs.fna inflated_3_seqs.fna
inflated_7_seqs.fna inflated_8_seqs.fna > denoised_seqs_2012.fna
  truncate_reverse_primer.py -f denoised_seqs_2012.fna -m Map_2012.txt -o
reverse_primer_removed_2012/

  #2013#
  denoiser.py -i IET0IJC04.txt -f split_library_output/4/seqs.fna --primer
TCCTCCGCTTATTGATATGC -v -o denoise/4 -c -n 8
  denoiser.py -i IET0IJC05.txt -f split_library_output/5/seqs.fna --primer
TCCTCCGCTTATTGATATGC -v -o denoise/5 -c -n 8
  denoiser.py -i IET0IJC06.txt -f split_library_output/6/seqs.fna --primer
TCCTCCGCTTATTGATATGC -v -o denoise/6 -c -n 8
  denoiser.py -i IET0IJC07.txt -f split_library_output/7/seqs.fna --primer
TCCTCCGCTTATTGATATGC -v -o denoise/7 -c -n 8
  denoiser.py -i IET0IJC08.txt -f split_library_output/8/seqs.fna --primer
TCCTCCGCTTATTGATATGC -v -o denoise/8 -c -n 8

  inflate_denoiser_output.py -c denoise/4/centroids.fasta -s
denoise/4/singletons.fasta -f split_library_output/4/seqs.fna -d
denoise/4/denoiser_mapping.txt -o inflated_4_seqs.fna
  inflate_denoiser_output.py -c denoise/5/centroids.fasta -s
denoise/5/singletons.fasta -f split_library_output/5/seqs.fna -d
denoise/5/denoiser_mapping.txt -o inflated_5_seqs.fna
  inflate_denoiser_output.py -c denoise/6/centroids.fasta -s
denoise/6/singletons.fasta -f split_library_output/6/seqs.fna -d
denoise/6/denoiser_mapping.txt -o inflated_6_seqs.fna
  inflate_denoiser_output.py -c denoise/7/centroids.fasta -s
denoise/7/singletons.fasta -f split_library_output/7/seqs.fna -d
denoise/7/denoiser_mapping.txt -o inflated_7_seqs.fna
  inflate_denoiser_output.py -c denoise/8/centroids.fasta -s
denoise/8/singletons.fasta -f split_library_output/8/seqs.fna -d
denoise/8/denoiser_mapping.txt -o inflated_8_seqs.fna
```

```
  cat FungusMap_4.txt FungusMap_5.txt FungusMap_6.txt FungusMap_7.txt
FungusMap_8.txt> Map_2013.txt
  cat inflated_4_seqs.fna inflated_5_seqs.fna inflated_6_seqs.fna
inflated_7_seqs.fna inflated_8_seqs.fna > denoised_seqs_2013.fna
  truncate_reverse_primer.py -f denoised_seqs_2013.fna -m Map_2013.txt -o
reverse_primer_removed_2013/

  ##################################### extract ITS2 region with ITSx
##############################################
  #2012#
  ITSx -i
reverse_primer_removed_2012/denoised_seqs_2012_rev_primer_truncated.fna -o
ITSx_output_2012
  #use grep to remove all non fungal sequences from the file
ITSx_output_2012.ITS2.fasta
  #restore the original QIIME headers to the ITSx output files
  perl restore_headers.pl denoised_seqs_2012_rev_primer_truncated.fna
ITSx_output_2012/ITSx_output_2012_ITS2_F

  #2013#
  ITSx -i
reverse_primer_removed_2013/denoised_seqs_2013_rev_primer_truncated.fna -o
ITSx_output_2013
  #use grep to remove all non fungal sequences from the file
ITSx_output_2013.ITS2.fasta
  #restore the original QIIME headers to the ITSx output files
  perl restore_headers.pl denoised_seqs_2013_rev_primer_truncated.fna
ITSx_output_2013/ITSx_output_2013_ITS2_F

  #combine two years seqs#
  cat ITSx_2012_output.ITS2_F.restored.fasta
ITSx_2013_output.ITS2_F.restored.fasta > ITSx_output_all_F.fasta

  ########################################## Denoise with USEARCH
#####################################################
  pick_otus.py -i ITSx_output_all_F.fasta -m usearch --word_length 64 -f
its_12_11_otus/rep_set/99_otus.fasta -g 1 -F intersection -o
ITSx_usearch_qf_intersection_0.99denoise -j 0.99

  ############################## CROP OTU picking at 97% similarity with
crop/intel/1.33################################
  CROP -i repr_set.fasta -o CROP.exact_otus -s -b 86 -z 882

  #Downstream processing of CROP output, 2849 OTUs #
  #Use TextWrangler to replace commas in CROP.exact_otus.cluster.list with tab
characters, creating CROP.exact_otus.cluster.tab.list
  #Merge CROP and USEARCH pipeline OTU maps (see
http://qiime.sourceforge.net/scripts/merge_otu_maps.html for what this means).
  merge_otu_maps.py -i
ITSx_usearch_qf_intersection_0.99denoise/ITSx_output_all_F_otus.txt,CROP.exact_o
tus.cluster.tab.list -o merged_otu_crop97_map.txt

  # making OTU tables #
  make_otu_table.py -i merged_otu_crop97_map.txt -o
merged_otu_table_crop97_notax.biom

  #nonsingleread #
```

```
  mkdir nonsingleread
  filter_otus_from_otu_table.py -i merged_otu_table_crop97_notax.biom -o
nonsingleread/otu_table_crop97_notax_nonsingleread.biom -n 1

  #Assign taxonomy by Claident#
  clidentseq blastn -strand plus -task blastn -word_size 9 end --
bdb=fungi_ITS_genus --method=NNC+QC --numthreads=6 CROP.exact_otus.cluster.fasta
retri_nncqc_neighb_seq.txt
  classigntax --taxdb=fungi_ITS_genus retri_nncqc_neighb_seq.txt
claident_nncqc_identresult.txt

  #add taxonomy into otutable#
  #perl file 'table_add_taxonomy_QIIME.pl' written by XXW and YCX
  perl table_add_taxonomy_QIIME.pl -i1 otu_table_crop97_notax_nonsingleread.txt
-i2 claident_nncqc_identresult_edited.txt -o otutable_claident_nncqc_tax.txt

  #add 'Unassigned' after otus failed to assign#

  #convert txt file to biom
  biom convert -i otutable_claident_nncqc_tax_edit2.txt -o
otutable_claident_nncqc_tax_edit2.biom --table-type="OTU table" --process-obs-
metadata taxonomy

  biom convert -i otutable_crop_claident_final.txt -o
otutable_crop_claident_final.biom --table-type="OTU table" --process-obs-
metadata taxonomy

  #taxonomy summary#
  summarize_taxa_through_plots.py -i otutable_claident_nncqc_tax_edit2.biom -o
otu_taxa_summary2 -m map_2yearsall.txt

  summarize_taxa_through_plots.py -i otutable_crop_claident_final.biom -o
otu_taxa_summary_final -m map_2yearsall.txt


  ############################## UCLUST OTU picking at 97% similarity V1
##############################################
  pick_open_reference_otus.py -i ITSx_output_all_F.fasta -r
its_12_11_otus/rep_set/97_otus.fasta -o ucrss_ITSx_all/ -p params_97.txt --
suppress_align_and_tree

  #params_97.txt as followed#
   assign_taxonomy:rdp_max_memory 20000
   pick_otus:enable_rev_strand_match True
   pick_otus:max_accepts 20
   pick_otus:max_rejects 500
   pick_otus:stepwords 20
   pick_otus:word_length 12
   assign_taxonomy:id_to_taxonomy_fp
its_12_11_otus/taxonomy/97_otu_taxonomy.txt
   assign_taxonomy:reference_seqs_fp its_12_11_otus/rep_set/97_otus.fasta
   beta_diversity:metrics bray_curtis

  # making OTU tables #
  #nonsingleread #
  mkdir nonsingleread
  filter_otus_from_otu_table.py -i otu_table_mc2_w_tax.biom -o
nonsingleread/otu_table_mc2_w_tax_nonsingleread.biom -n 1
```

```
  # convert biom to txt (qiime 1.8)#
  biom convert -i otu_table_mc2_w_tax_nonsingleread.biom -o
otu_table_mc2_w_tax_nonsingleread_tax.txt -b --header-key taxonomy

  #delete 1: the samples are not sequences in batch, 2: delete OTUs counts
sum=0/1
  #convert back to biom
  biom convert -i otutable_uclust_tax_final.txt -o
otutable_uclust_tax_final.txt.biom --table-type="OTU table" --process-obs-
metadata taxonomy

  #taxonomy summary#
  summarize_taxa_through_plots.py -i otutable_uclust_tax_final.txt.biom -o
otu_taxa_summary2 -m map_2yearsall.txt
```