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Higher fungal diversity in dead wood is correlated with lower CO_2 emissions in a natural forest

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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 results are consistent with the theory of interference competition and with the results of published experiments using low-diversity, laboratory-inoculated wood, and we extend those results to a high-diversity, natural system for the first time. High levels of saprotrophic fungal biodiversity might be providing globally important ecosystem services by maintaining dead-wood habitats and by slowing atmospheric contribution of CO_2 from the world's stock of decomposing wood.

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 rate variation. 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

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much higher species diversity, more complex assembly histories, and selective faunal feeding on decomposers[14, 15, 16]. Thus, it is important to examine the relationships 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 (*Lithocarpus chintungensis* LC [Fagaceae], *L. xylocarpus* LX, and *Schima noronhae* SN [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 natural fungal community variation can explain variation in decomposition 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 emissions, emissions declined with fungal diversity in all wood species (Fig 1C, F, R^2 = 26% to 44%).

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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 top).

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.

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 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 S2). 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. the 'pure-diversity' effect). For two tree species, LC and LX, λ always took values nearly equal to zero (Table 2). For the third tree species SN, λ was also nearly zero in June 2012 but took intermediate values in

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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 also the only ones to line up along the CO_2 emissions gradient (except the lowest diversity samples). Not surprisingly, conventional community-analysis tests returned the same conclusion: variation in community composition is not explained by CO_2 emissions (Supporting Information S3).

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 important decomposing-wood habitats 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

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community-composition differences for logs that differed in remaining undecomposed weights.

Importantly, none of those three field studies measured fungal diversities and CO_2 emission rates concurrently, as we did here (Fig. 1). We observe that relationships between CO_2 emission 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, that fungal community composition 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 decomposer species drives positive relationships between diversity and soil organic matter decomposition.

A plausible biological mechanism explains why wood decomposition should slow with fungal diversity. Interference competition has long been predicted to evolve when niche overlap is high and the disputed resource is valuable[36]. In forests, decomposing wood resources are available to many fungal species, and aggressive interactions are indeed observed among these fungi[37]. Elsewhere, it has been shown that interference competition reduces virulence (= host consumption rate) in endosymbioses[38.39,40] and productivity in

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bacterial communities[41,42]. Consistent with those findings in other contexts, interference competition can also explain why fungal biomass has been found to explain variance in wood mass loss[9]. When a piece of wood is colonized by many fungal species, the hypothesized higher levels of interference competition would result in less wood converted into fungal biomass (or CO_2). High niche overlap is also consistent with the observed 'pure diversity' effect of fungal diversity on emissions (Table 2, S2) since any species should fight all others (note, however, that we could not test for community composition effects at higher taxonomic levels, see Maherali & Klironomos[43]). Thus, theory suggests that the arrow of causation can be drawn in the direction of fungal diversity driving decomposition rate. Finally, interference competition results in competitive exclusion, which will cause community composition to change over time. This means that measures of fungal diversity made after years of decomposition are unlikely to explain final variance in decomposition.

Possible errors and future experiments. - One potential source of error is that metabarcoding provides only approximate estimates of species frequencies[22, 23], yet Shannon and Simpson indices, both of which discount rare species, were able to explain variation in CO_2 emissions here. There are two likely and non-exclusive explanations. (1) Low-read ('rare') OTUs were more likely to be sequence artifacts from the metabarcoding pipeline and thus 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 from metabarcoding were positively correlated, suggesting that low-read OTUs represent low-biomass species, which could have little 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 correlation between fungal diversity and CO_2 emissions rate (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'

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effects of fungal communities on emissions (Table 2), whereas this alternative explanation invokes a successional sere and thus predicts compositional effects. We suggest other long-term experiments in which sterilized and even-aged wood pieces are allowed to be colonized and sampled for CO_2 emissions rates and fungal diversity over many years in the field.

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

Methods

Site description. – This study was conducted in the Ailao Mountains National Nature Reserve, Yunnan, China, which preserves the largest area of undisturbed, subtropical moist forest in China and has a substantial pool of woody debris (branches and logs, 74.9 x 10^3 kg ha^{-1}, Ref. 47). The study site was at an elevation of 2476 m, about 2 km north of the Ailao Field Station for Forest Ecosystem Studies (24.533 ºN, 101.017 ºE), and receives 1840 mm annual average precipitation. The climate is monsoonal with distinct cool/dry (November to April) and warm/wet (May to October) seasons[48]. Annual mean air temperature is 11.3 ºC with monthly means ranging from 5 to 16 ºC. Surface soils (0 to 10 cm) of the area are Alfisols with pH of 4.2 (in water). The surficial organic layer is 3 to 7 cm deep[49]. The study

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site is a broad-leaved evergreen subtropical forest, with the canopy dominated by *Lithocarpus chintungensis, Rhododendron leptothrium, Vaccinium ducluoxii, Lithocarpus xylocarpus, Castanopsis wattii, Schima noronhae, Hartia sinensis*, and *Manglietia insignsis*[50].

Experimental setup. – At our site, most woody debris comes from *Lithocarpus chintungensis*, (LC), *Lithocarpus xylocarpus* (LX), and *Schima noronhae* (SN), so we only examined those three species. In early 2010, branches from these three species, already decomposing on the forest floor, were identified to species by a botanist from the Xishuangbanna Tropical Botanical Garden, collected, and cut into a total of 320 wood pieces, sized to fit a field-respiration chamber (*ca*. 10 cm diameter and 20 to 30 cm length), tagged, weighed, and measured for size and decay class (further details in *Liu et al.*[8]). The three decay classes were DKC1 = a knife could not penetrate, DKC2 = knife could slightly penetrate with appreciable resistance, DKC3 = knife could deeply penetrate with little resistance[51]. We used similar-sized pieces to control for potential effects of wood size on 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 shown 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:

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

Where ΔCO_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. – From the larger ongoing study, 65 wood pieces were selected to represent the three species and decay classes equally. Within each of those groups, selections included pieces that previously followed group means through time, and others that had high variations in CO2 emission during 2010-2012. Pieces for each of those subgroups were selected at random from the larger study. This stratified random sampling ensured that all 9 groups and the full range of CO2 emission variations were represented in the metabarcoded samples. Those wood pieces were collected in September 2012, and 59 of those samples were recollected in June 2013 (6 samples were not relocated in June 2013, having lost their tags or been buried under new litter). We chose September and June because CO_2 emissions from wood are typically high in June and lower in September, despite similarly warm temperatures and high moisture availability[8] (Fig. 1 top). Superficial litter and bark were removed from each wood piece with a flame-sterilized knife before drilling. An electric drill with a flame-sterilized, 11-mm drill bit was used to extract wood powder at three holes located near the ends and the middle of each wood piece. Each sample consisted of ~5 cm^3 pooled material from those three holes, collected onto aluminum-foil sheets, and then immediately stored in 50 ml tubes and frozen at -20 ºC for 2-3 days until transport on ice packs to our laboratory 10 hr away, where they were stored at -40 ºC until extractions.

Comparing cumulative CO_2 emissions and gravimetric weight losses. – Sixty-four additional wood pieces (*i.e.* not used for metabarcoding) were retrieved from the field in April 2013 to test the extent to which CO_2-emissions-estimated mass loss (averaged over the measurements taken from the year 2010 experiment start) accurately estimated directly measured mass loss (gravimetric weight loss). In the laboratory, these wood pieces were

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re-measured for volume (as above), and twenty-two wood pieces exhibiting >15% volumetric weight loss since the start of the experiment, indicating substantial fragmentation in the field, were excluded from the analysis. The remaining wood pieces were dried at 70 ºC to constant weights and then individually weighed to the nearest 0.1 g on an electronic balance. CO_2-estimated mass loss was positively correlated with gravimetric loss (linear regression, Gravimetric loss in grams over 3 years = 30.7 + 0.741 * CO_2-based-decomposition Cd (R^2 = 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 * Cd (R^2 = 0.592, n = 13, p <0.01), for DKC1, 2, and 3, respectively. Inspection of scatterplots (Supporting Information S4) revealed that the main discrepancy was that CO_2-emission mass-loss estimates slightly underestimated small mass losses.

DNA extraction, PCR, and 454 pyrosequencing of ITS2 amplicons. – Total DNA was extracted from each sample of wood powder by adding 10 mL CTAB buffer (2% cetyl trimethyl ammonium bromide, 50 mM NaCl, 5 mM EDTA, 10 mM Tris, pH 8) and 20 µL β-mercaptoethanol per 5 cm^3 of sample, homogenizing with a TH-02 homogenizer (Omni International, Kennesaw, GA USA) for 5 min at room temperature, incubating at 65 ºC for 1 hour, centrifuging at 4,000 rpm for 1 min. After centrifugation, the supernatant was transferred to new Axygen® 2.0 mL microcentrifuge tubes and extracted using one volume of chloroform by vortexing for 20 min and centrifuged at 4 ºC, 12,000 rpm for 10 min. The supernatant was then transferred to new microcentrifuge tubes and precipitated with 1.5 volumes of precooled isopropanol at -20 ºC overnight. After centrifugation at 4 ºC, 12,000 rpm for 20 min, the precipitate was washed with 70% ethanol and dissolved in 100 µL TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). DNA was purified by using QIAquick PCR Purification Kit. The quantity and quality of purified DNA was assessed with a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). Samples were PCR amplified using the forward primer ITS3_KYO2 5'-GATGAAGAACGYAGYRAA-3' [18] and reverse primer ITS4 5'-TCCTCCGCTTATTGATATGC-3' [53]. The standard Roche A-adaptor and a unique 10 bp MID (Multiplex Identifier) tag for each sample were attached to the forward primer. PCRs were performed using approximately 10 ng DNA in a 20 µL reaction mixture containing 2 µL of 10X buffer (Mg^{2+} Plus), 0.02 mM dNTPs, 20 µg Bovine Serum Albumin, 1 µL DMSO, 0.4 µM of each primer, and 0.5 U HotStart Taq DNA

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polymerase (TaKaRa Biotechnology Co., Dalian, China) under a temperature profile of 95 ºC for 10 min, followed by 35 cycles of 94 ºC for 20 sec, 47 ºC for 30 sec, and 72 ºC for 2 min, and final extension at 72 ºC for 7 min. For pyrosequencing, PCR products were gel-purified using Qiagen QIAquick PCR purification kit, quantified using the Quant-iT PicoGreen dsDNA Assay kit (Invitrogen, Grand Island, NY, USA), pooled and A-amplicon-sequenced on a Roche GS FLX (Branford, Connecticut, USA) at the Kunming Institute of Zoology.

Bioinformatic analyses. – The sequences obtained were run through a pipeline for quality control, denoising and chimera removal, OTU-picking and taxonomic assignment. Quality Control: Header sequences and low-quality reads were removed from the raw output in the QIIME 1.8.0 environment (*split_libraries.py*: -l 100 -L 500 -H 30)[54]. The 65 samples collected in 2012 had been sequenced on four 1/8 regions, producing 679,361 raw reads and 525,679 post-quality-control reads (mean read length 286 bp). The 59 samples collected in 2013 had been sequenced on three 1/8 regions, producing 327,226 raw reads and 256,996 post-quality-control reads (mean read length 308 bp). These amplicon lengths were consistent with the expected mean length for ITS2 (327.2 bp, SD=40) reported by Toju *et al*.[18]. *Denoising and chimera removal: Denoiser* in QIIME[55] was used to remove characteristic 454 sequencing errors. Next, ITSx 1.0.3[56] was used to extract the variable ITS2 region from the whole reads (i.e. conserved 5.8S and LSU flanking sequences were stripped) and to remove non-fungal-ITS reads. The extracted sequences were clustered at 99% similarity with USEARCH v7.0.1090[57] to remove replicate sequences and chimeras. *OTU-picking and taxonomic assignment*: We used two methods to cluster the reads into OTUs. First, we used a reference-based method in QIIME (*pick_open_reference_otus.py*: max_accepts 20 max_rejects 500 stepwords 20 length 12 –suppress_align_and_tree) in which reads were first clustered by matching at 97% similarity to the UNITE 12_11 fungal database[58], which itself had previously been clustered at 97% similarity for use within QIIME. Unassigned reads (the vast majority) were then clustered *de novo* using the *uclust* option at 97% similarity, producing 1,807 OTUs in total. For these latter OTUs, we attempted to assign taxonomies using QIIME's *assign_taxonomy.py* against the UNITE database. Second, we performed *de novo* 97%-similarity clustering with CROP 1.33[59], producing 1,565 OTUs. We assigned taxonomies against Genbank using the NNCauto and QCauto methods in Claident[60].

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Sequence data will be deposited at datadryad.org (doi: to be assigned) and in GENBANK's Short Read Archive (Accession number: PRJNA252416). An example bioinformatic script will also be deposited at datadryad.org (doi: to be assigned).

Statistical analyses. – Analyses were performed using *vegan* 2.0-10[61] and *mvabund* 3.8.4[62] in *R* 3.1.0[63]. The HTML outputs of the R scripts are deposited at datadryad.org (doi: to be assigned). From both the OTU tables generated (CROP and *uclust*, see *Bioinformatic analysis*), we deleted one SN wood piece that had only 35 reads and then split the tables by wood species (LC, LX, and SN) and sample time (September 2012 and June 2013). We then generated a second pair of OTU tables by rarefying to the lowest read number per wood piece in the dataset (*rrarefy()* in *R*). Thus, for each wood species and sampling time, we have four OTU tables: CROP/non-rarefied, 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 new 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 S2). In Supporting Information S3, we also

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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.

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Figure legends

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

Figure 2. Correspondence analysis ordinations of fungal communities, by wood species and sampling date. Point size is scaled to CO_2 emissions, and the gradient represents fungal Shannon diversity. In all ordinations (A-F), CO_2 emissions decrease with higher fungal diversity (point size decreases up the gradient, echoing Fig. 1). Also evident is that the lower diversity wood pieces are compositionally very dissimilar to each other and to the higher diversity wood pieces. Left-hand column (A, C, E). June 2012 CO_2 vs. September 2012 fungal diversity. Right-hand column (B, D, F). June 2013 CO_2 vs. June 2013 fungal diversity. A-B. *Lithocarpus chintungensis*. Note that the label for point 14 at the top of A is obscured by the small point size. C-D. *L. xylocarpus*. E-F. *Schima noronhae*. Shown here are the non-rarefied datasets clustered using CROP (see Methods). Rarefaction or using *uclust*-clustering does not change the results (Supporting Information S3).

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 Materials and 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%		

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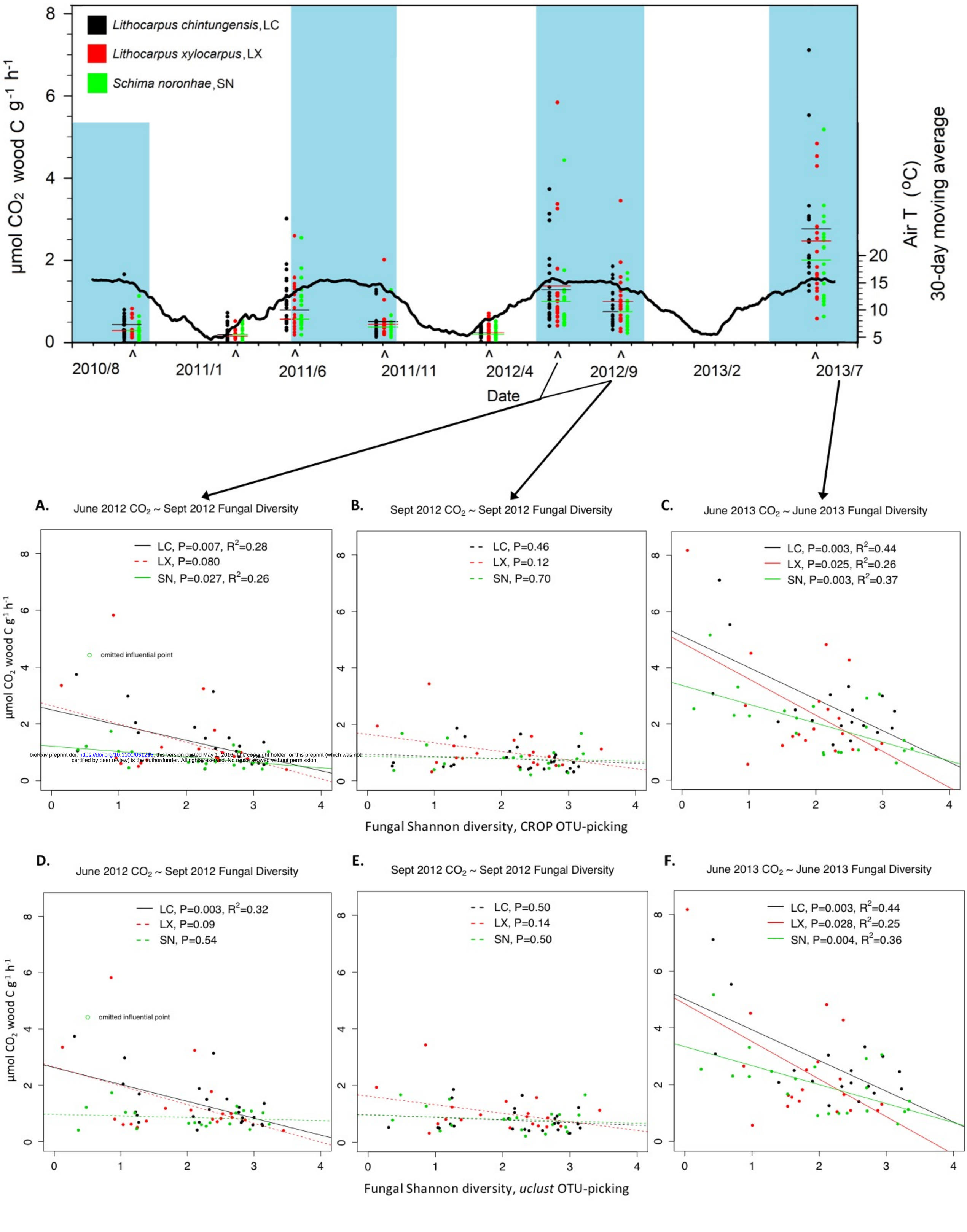
Table 2. Estimation of the contribution of fungal community composition to CO_2 emissions, using the method of Sandau *et al*. (2014). The generated parameter λ varies between 0 and 1, with 0 indicating that variation in composition does not explain variation in emissions. Composition only contributes to explaining variation in one tree species in one sampling date (SN, June 2013). Conventional community analysis (Supporting Information S3) also finds a possible 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



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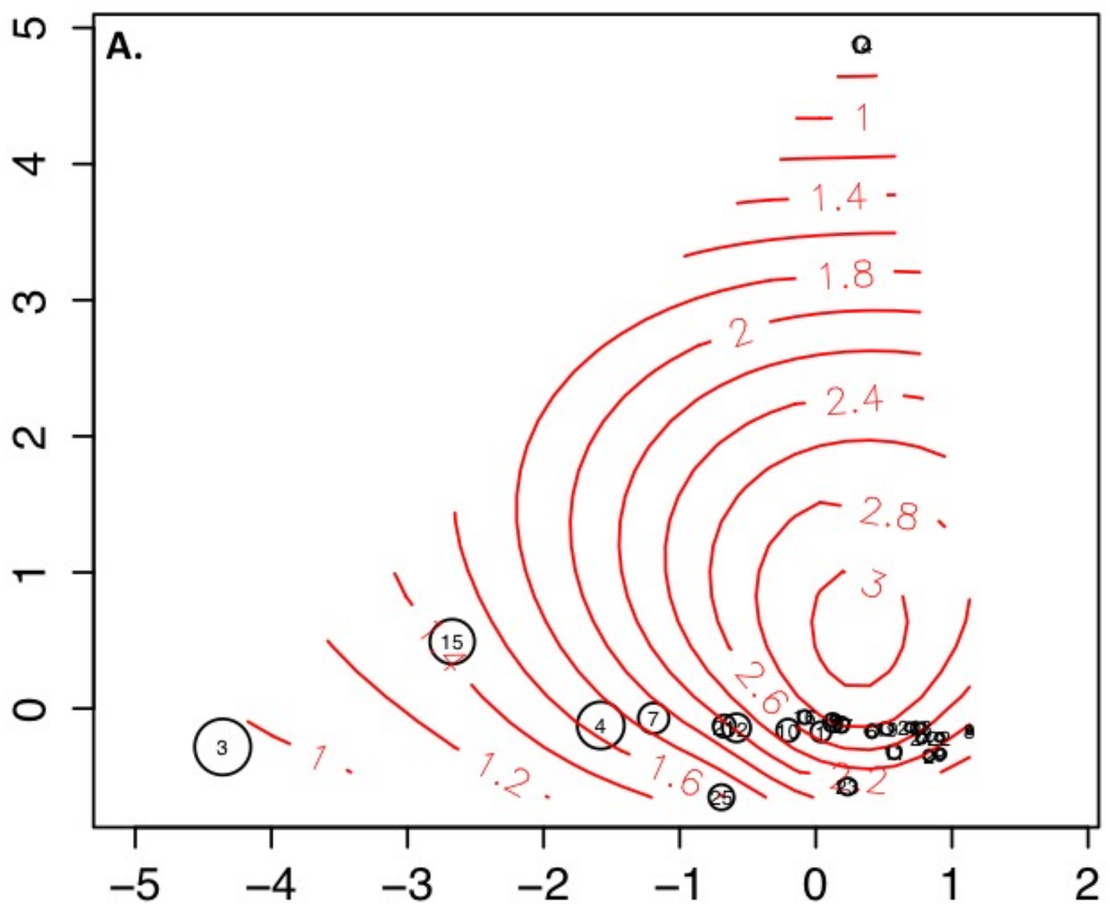
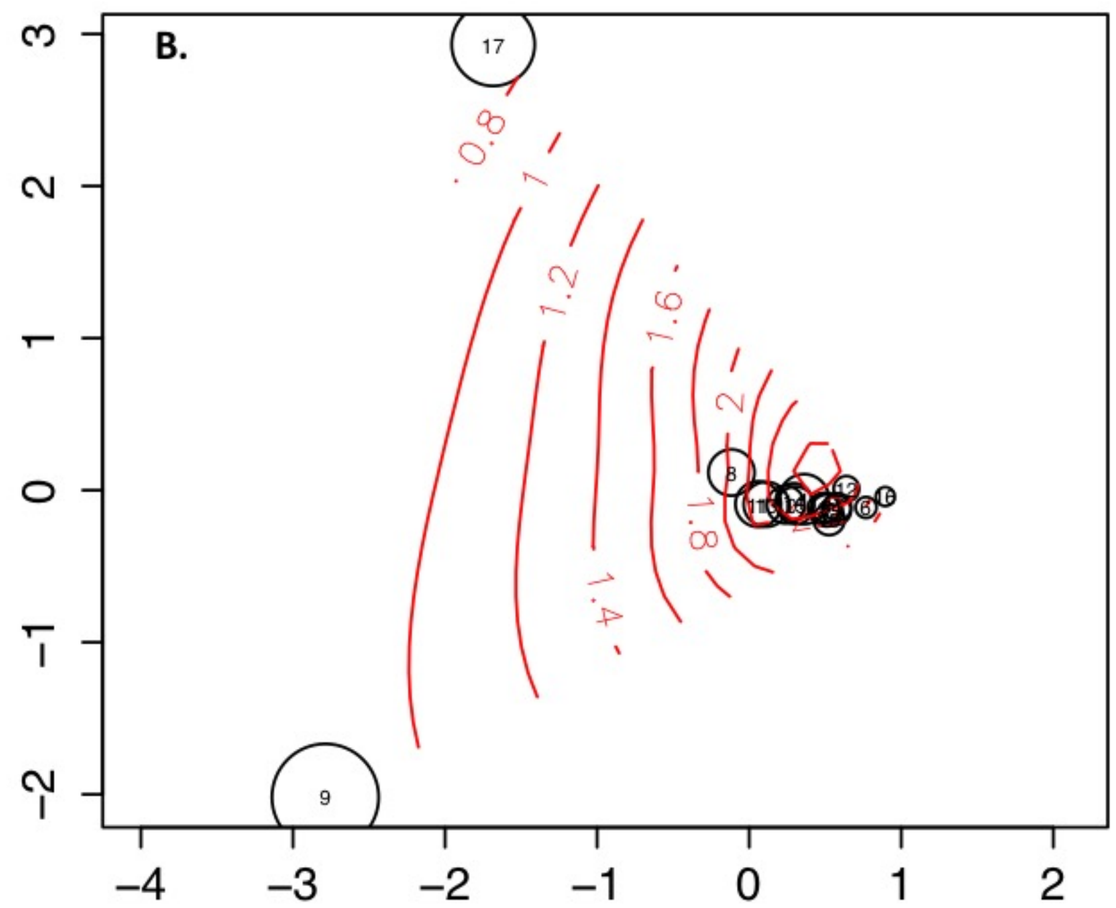
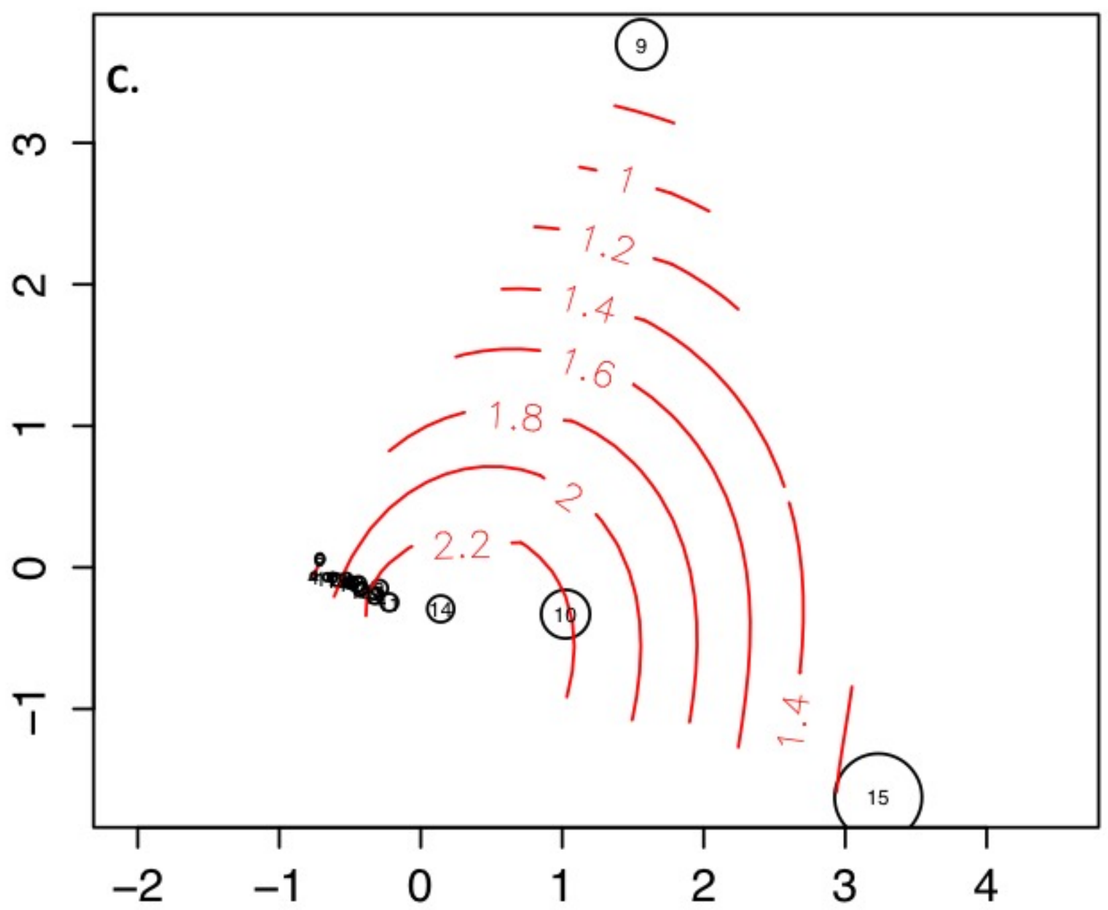
LC, June 2012 CO_2 ~ Sep 2012 Shannon



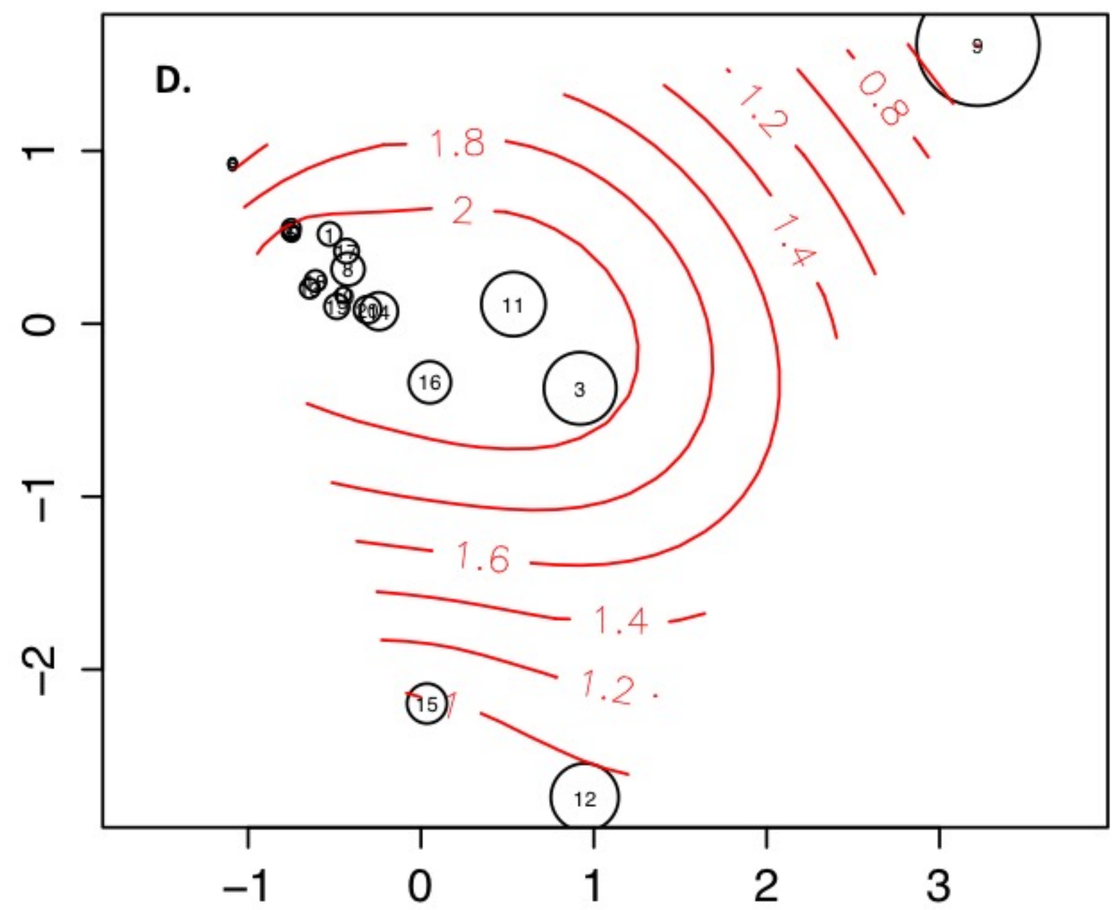
LC, June 2013 CO_2 ~ June 2013 Shannon



LX, June 2012 CO_2 ~ Sept 2012 Shannon



LX, June 2013 CO_2 ~ June 2013 Shannon



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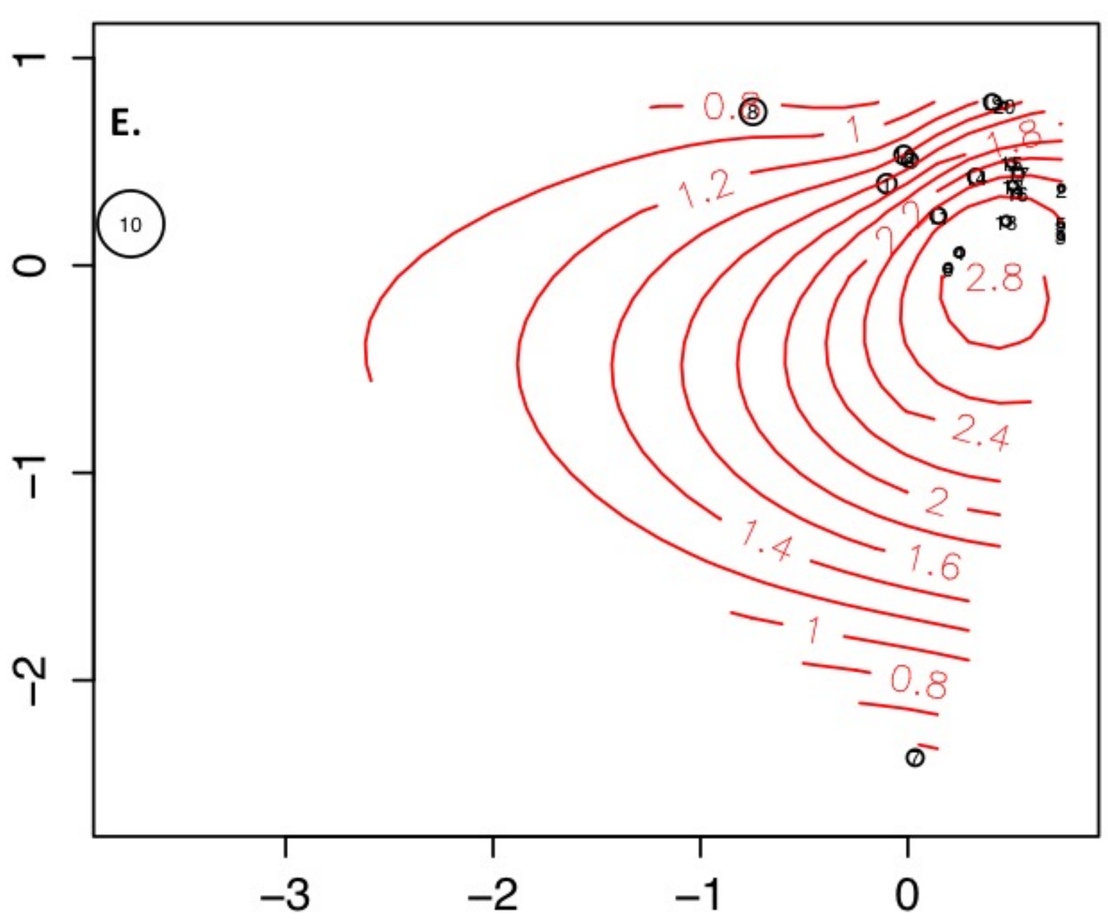
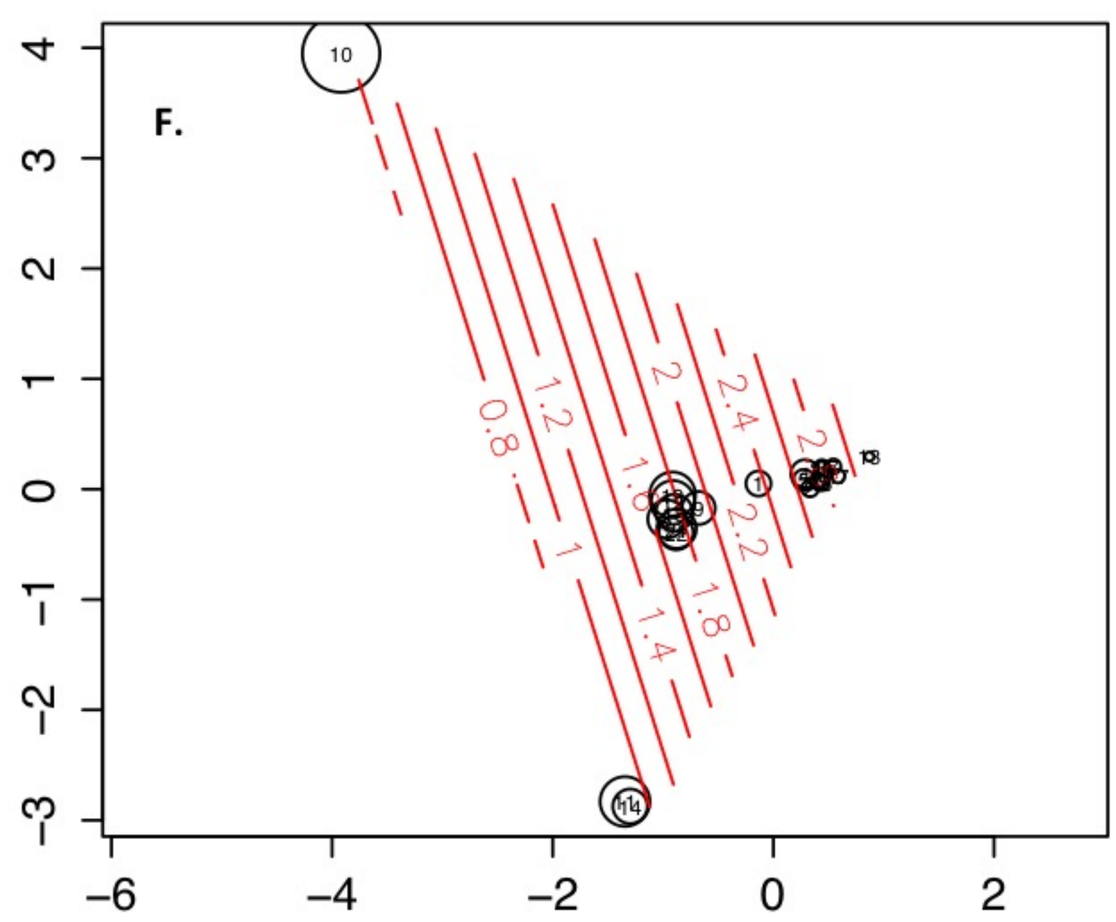
SN, June 2012 CO_2 ~ Sept 2012 Shannon



SN, June 2013 CO_2 ~ June 2013 Shannon