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探討暖化下地上部與地下部的生物交互作用-路徑分析與直接、間接交互作用之量化

Identify the Path Structure and Quantify Direct and Indirect Effects in an Above-below-ground System under Warming

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本論文曾子榮君（R00B44020）在國立臺灣大學生態學與演化生物學研究所完成之碩士學位論文，於民國103年6月3日承下列考試委員審查通過及口試及格，特此證明

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摘要

暖化可能會影響地上部地下部的生物交互作用，改變直接或間接的交互作用的強度，但是相關的研究卻相當缺乏。為幫助了解暖化對生物交互作用的影響，並量化其中的直接和間接的生物交互作用，本研究在生長箱中建立地上部地下部生物交互作用的系統，藉由調控溫度來模擬正常(日均溫 22.5℃，夜均溫 18.5℃)及暖化(日夜均溫各加 4℃)的兩個情景。本研究在各溫度下設立了四種處理:控制組(只有植物竹仔菜 *Commelina diffusa*)、蚯蚓組(植物加皮質遠環蚓 *Amynthas corticis*)、蚜蟲組(植物加棉蚜 *Aphis gossypii*)，及蚯蚓蚜蟲組(加入植物、蚯蚓、蚜蟲)。記錄植物的形態變化、土壤的物化性質、落葉分解速度、蚯蚓的重量、及蚜蟲的族群增長，以便探討及量化各因素間的直接或間接關係。資料分析以冗餘分析來探討植物受蚜蟲蚯蚓及暖化的影響，以貝氏階層模式來量化直接效應，以導向分離法及中介分析來判別並量化可能存在的間接效應。研究結果顯示，蚯蚓及蚜蟲對植物的影響強度類似，但是方向相反；而暖化雖然對植物有很強的影響，但此效應隨著時間而呈線性地下降，且不會影響蚯蚓及蚜蟲對植物的效應。蚯蚓、蚜蟲及暖化處理的交互作用並不影響系統中的任何特質，代表這三個因子的影響是加成性的。中介分析顯示出植物氮含量及土壤物理性質是這系統中的中介因子，分別是蚯蚓能藉由增加植物氮含量而間接增加蚜蟲的族群量，以及暖化和蚯蚓處理能改善土壤的物理性質，進而間接促進植物的生長（節數的增加）。整體而言，這試驗顯示出暖化可以透過直接及間接的路徑，進而影響地上部與地下部的系統。

關鍵字：地上部地下部交互作用、暖化、蚯蚓、蚜蟲、中介分析

Abstract

Climate warming could affect the interactions between above- and below- ground biota and change the strength of direct or indirect effects, but relevant studies are sparse. To better understand above-below-ground interactions under warming, this study examined a system including the plant *Commelina diffusa*, the aphid *Aphis gossypii*, and the earthworm *Amynthas corticis* under 2 scenarios: (1) normal (day and night temperature at 22.5 and 18.5^{o}C, respectively) and (2) warming (a 4 ^{o}C increase in day and night temperature). Each scenario included four treatments: a) control treatment (plants only), b) earthworm treatment (plants, earthworms), c) aphid treatment (plants, aphids), and d) earthworm–aphid treatment (plants, aphids, earthworms). To qualify and quantify the direct and indirect effects in this system, I measured the traits of plants, earthworms, aphids, litter, and soil. Data analyses were conducted by (1) redundancy analysis for exploring the general patterns of plant traits in response to the treatment factors (earthworm, aphid, and warming); (2) Bayesian hierarchical modelling to quantify the direct effects in the system; (3) d-sep test and mediation analysis for identifying and quantifying the indirect effects. The results showed that (1) the effect sizes of earthworm and aphid treatments on plants were similar but different in direction, and showed a non-contingent response to warming; (2) the effect of warming treatment on plants was strong but decreased linearly with time; (3) there were no interactions among aphid, earthworm, and warming treatments in this system; thus, the effects of these 3 factors were additive. In addition, I found 2 indirect effects in this system, suggesting plant nitrogen content and soil physical property as mediators in above-below-ground interactions: (1) earthworm treatment increased aphid population by increasing plant nitrogen content, and (2) warming and earthworm treatments increased plant growth (i.e. plant node number) by modifying soil physical property. Overall, this study reveals that warming can affect an above-below-ground system via direct and indirect pathways.

Keyword: above-below-ground interaction, climate warming, aphid, earthworm, mediation analysis

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Introduction

Global warming is a great threat to many ecosystems on earth. Numerous studies have suggested that warming could impact ecosystems by affecting individual metabolism, behavior, population dynamics, migration, phenology, species distribution (e.g. poleward shift), community structure (e.g. reshuffling), ecosystem functions (e.g. nutrient cycling), etc. (Walther *et al.* 2002,Harley *et al.* 2006, Parmesan *et al.* 2006, Bonan 2008, Blois *et al.* 2013). As climate warming is expected to intensify significantly by 2100 (IPCC AR5 2013), there is an urgent need to understand how ecosystems will respond to upcoming warming. To improve our capability to understand ecosystem response, we need to take into consideration both direct and indirect biotic interactions under warming; otherwise, our predictions could be misleading (Davis *et. al.* 1998, Tylianakis *et al.* 2008). For example, it could be biased to directly predict species response to warming by climate envelope model, which solely relies on environmental variables (such as temperature and rainfall) to define species tolerance and predict their range after climate warming. In this model, biotic interactions indirectly affected by warming are ignored, although they could be important. In order to understand how species will be affected by warming, we should examine both the direct impacts from change in environmental variables and the indirect impacts from change in interactions with its dependent species (such as its preys, predators, and hosts) (Gilman 2010).

In line with the need to understand biotic interactions under warming, the number of relevant studies has increased in recent years (Blois *et al.* 2013). However, most studies of warming impact on species interactions in terrestrial systems have mainly focused on aboveground systems (Barton 2010, Cahoon *et al*. 2012, Dyer *et al*. 2013), and therefore we still know little about the warming impact on below-ground systems or above-below-ground systems as a whole. This knowledge gap impedes our ability to understand ecosystem responses under warming. In specific, our understandings of ecosystem responses could be misleading if they are mainly based on above-ground studies, because above- and below-ground systems may respond to warming in different directions, and/or these responses may interact with each other. For example, warming could shift ecosystem functions mainly by its dramatic impacts on belowground biota, but not by its negligible impacts on aboveground system (Briones *et al.* 2009). Therefore, predictions based only on above-ground systems may miss the whole picture. Furthermore, it is critical to understand the interactions between above- and below-ground systems regarding to the recycling of carbon in terrestrial ecosystems. Climate warming may directly and indirectly affect decomposition (below-ground) or photosynthesis (above-ground) and then interfere with the feedback to carbon pool in atmosphere (Davidson *et al.* 2006). A study of warming impact on an above-below-ground system, as an interface of recycling of carbon in terrestrial ecosystem, is required to improve our understanding.

Plants play as a key mediator of above-below-ground interactions (Wardle *et al.* 2004). These interactions in general can be separated into direct and indirect pathway to plants, depending on how above- or below-ground species acquire plant resource. In the direct pathway, species such as herbivores and parasites acquire their needs directly from "living plants". These species may then interact with species at higher trophic levels (e.g. predators), affect plant performance, or influence below-ground species (e.g. root herbivores) (McKenzie *et al.* 2013). In the indirect pathway, above- or below-ground species use plant detritus as energy resource. In fact, the majority of energy (> 75%) plants fix eventually becomes detritus, which may be utilized by decomposers (M.J. Swift, *et al.* 1979). Interactions between direct and indirect pathways or feedbacks within each pathway may occur, complicating above-below-ground interactions. For example, herbivores may change plant condition (direct pathway) and consequently affect the quality and quantity of plant detritus and then associated decomposers (indirect pathway) (Ibanez *et al.* 2013, Katayama *et al.* 2013). Besides, feedbacks within a pathway could exist, such as when above-ground herbivores affect below-ground herbivores, which may in turn affect above-ground herbivores (McKenzie *et al.* 2013). The reverse (below-ground herbivores' feedback to themselves) could happen as well.

One way to quantify (standardize) the diverse interactions in above-below-ground systems (mentioned above) is to use interaction strength (i.e. effect size, O'Connor 2009, Hoekman 2010). Accordingly, exploring how interaction strength changes with rising temperature will likely produce promising insights for helping predict community responses to climate warming. The interaction strength (effect size) of species interactions could increase, decrease, or remain unchanged under warming scenario, and this could lead to very different implications. Studies have suggested interaction strengths could depend on different contexts (e.g. ecosystem type or species combination). For example, in an aquatic system, warming could strengthen top-down effects but had relatively neutral impact on bottom up effects (Hoekman 2010). In terrestrial system, interactions between above- and below- ground community were context-dependent (such as fertility, spatial, and temporal scales) (Wardle *et al*. 2004, Bardgett *et al*. 2005). In other words, the interaction strengths in above- below-ground systems could be affected by many different factors, and it is likely that interaction strengths in above-below-ground systems will differ in the context of climate warming.

To qualify (e.g. direct or indirect interactions) and quantify (e.g. interaction strength) the above-below-ground interactions under ambient and warming environment, I constructed an above-below-ground model system in growth chambers with 2 temperature settings: ambient and warming (+ 4 ℃) treatments. A 4 ℃ increase in global temperature is among possible warming scenarios by 2100 (IPCC AR5 2013). This model system contained 3 species: epigeic earthworm (*Amynthas corticis*; below-ground decomposer), plant (*Commelina diffusa*; mediator of this above-below-ground system), and cotton aphid (*Aphis gossypii*; above-ground herbivore). All of them are very common and representative species in low altitude Taiwan (personal observations). Past studies provided some guidance for species interactions in this above-below-ground system. For example, earthworms were well-recognized for their role as below-ground decomposers and ecosystem engineers (organisms with the ability to modify physical properties of habitat and then affect other species) (Jones, *et al*. 1994, 1997; Edwards 1996). Therefore, I expected that they would increase the recycling of nutrients and modify the soil physical properties, and then indirectly benefit the performance of the plant *C. diffusa*. On the other hand, cotton aphids may directly deprive nutrients from *C. diffusa* (Ebert *et al*. 1997). The two potential counter forces on *C. diffusa* (positive from earthworms and negative from aphids) were compared for their effect sizes on plant traits, and their interaction (additive, over-additive, or under-additive), if applicable, were analyzed. Previous studies did not provide a consensus about the interaction between earthworms and aphids. Earthworms may have positive effects on plants but either positive or negative effects on aphids (Eisenhauer *et al.* 2010, Wurst & Forstreuter. 2010, Johnson, *et al*. 2011). Whether the interactions between earthworms and aphids will change under warming remain unclear.

To explicitly measure the interaction strength (i.e. effect size) of direct and indirect effects in this system, this study applied statistical approaches including Bayesian hierarchical modeling and mediation analysis. Bayesian statistics could measure the precise effect size of direct effects, while mediation analysis (by potential outcome approach) could address the effect size of indirect effects (MacKinnon *et al.* 2007). Furthermore, the interaction strengths were calculated as Paine's index (Berlow *et al.* 2004, Timothy & Mark 2005).

Research objectives

This study was aimed to qualify and quantify the direct and indirect interactions in an above-below-ground community (aphids, plants, earthworms) under ambient and warming environment. This study tried to answer these 4 research questions: (1) In general, how plant traits were affected by warming, aphid, and earthworm treatments? (2) Did interactions among these three treatments exist? (3) How strong was the direct effect of each treatment on different traits? (4) Did indirect treatment effect exist in this system? Knowledge gained from this study would improve our ability to understand warming impact on communities that include both above- and below-ground components.

Material and methods

Above-below-ground system setup

The above-below-ground system in this study included 3 focal species: epigeic earthworm (*Amynthas corticis*; below-ground decomposer and ecosystem engineer), plant (*Commelina diffusa*; mediator of this above-below-ground system), and cotton aphid (*Aphis gossypii*; above-ground herbivore), all of which are common in lowland Taiwan. This study constructed an above-below-ground system in growth chambers with 2 temperature settings: ambient and warming (+ 4 ℃, a possible scenario by 2100 based on IPCC AR5). Under each temperature setting, there were four treatments based on a combination of the focal species: a) control treatment (plants only), b) earthworm treatment (plants, earthworms), c) aphid treatment (plants, aphids), and d) earthworm-aphid treatment (plants, aphids, earthworms). Each treatment had 12 replicates, making a total of 96 replicates in this study.

To avoid idiosyncrasy, this study collected plants and aphids together from 4 sites around North Taiwan: National Taiwan University (NTU) (25°01’11.2” N, 121°32’37.5” E), Tu-Cheng (T) (24°57’51.3” N, 121°26’50.0” E), Gu-Ting Riverside Park (G) (25°01’09.6” N, 121°31’20.6” E), and Hua-Jiang Riverside Park (W) (25°00’51.4” N, 121°29’29.3” E). Their stocks were then established and maintained separately in a common garden in NTU for at least 4 months before the experiment started. This would give them time to acclimate to the laboratory settings and produce enough ramets or individuals for experiments. Due to logistic constraints, adult earthworms were collected mainly from site T by hand-sorting method about 2 weeks before the experiment started. Approximately 500 kg of soil (yellow soil, clay loam) from site T was excavated, air-dried, sieved to < 2 mm to remove larger debris and initial soil structure (massive), and then modified by mixing additional organic matters (plant debris) to 1% w/w. The mixing of organic matters followed the protocol of local farmers. This study set up a total of 96 pots (the height, width, and length of each cuboid pot are 12.0 cm , 13.0 cm , and 31.5 cm, respectively) in two growth chambers (ambient vs. warming), with 48 pots in each of 2 experimental rounds due to space constraint (Figure 1). Each pot was filled with 3.2 kg prepared soil, supplied with 400 ml water, and covered with 10 g litter (mainly composed of *Ficus benjamina*) on the surface (10 ~30 g litter in the volume of soil of each pot was reasonable based on a previous field survey). In order to prevent earthworms and aphids from escaping, each pot was covered by silk screen (0.152 mm), which was supported by 6 plastic sticks (35 cm tall out of the soil ground), and the hole at the bottom of each pot was covered by nylon mesh (1 mm) with silicon glue. Plants and aphids from all collection sites were evenly distributed to each treatment and then randomly assigned to pots in that treatment. Based on the long term climate data (1981 - 2010) from the Central Weather Bureau of Taiwan, the average temperature from February to May in north Taiwan was 20.5℃ (22.5℃ at day and 18.5℃ at night), which would be the temperature setting of ambient scenario this study. The temperature for warming scenario in this study would be set at 24.5℃ (26.5℃ at day and 22.5℃ at night). Except for temperature, each scenario (growth chamber) had the same setting for other environmental factors: 12 hours for day period, 160~200 ppfd (photosynthetic photon flux density) for light intensity, and 75% for relative humidity.

Experimental procedures and trait measurements

Before the start of each experimental round (2 rounds in total), stems containing one node were cut down from individual plants that had been collected from each field site and then kept in a common garden. Six stems that successfully germinated (vegetative reproduction) one week later were transplanted to each pot in growth chambers (96 pots in total in this study). Small stocks of aphids collected from each field site were also established in each chamber for acclimation before the beginning of experiments. All pots were watered 400 ml once a week in the first 3 weeks to keep the new soil moist enough for young plants, and then 200 ml once a week afterward. In the second week, 6 stems were trimmed to 5 in each pot (within the reasonable density of field plants), ensuring that this study had five healthy individual plants each pot. In the second week, this study also added earthworms (2 weighed adults) and/or aphids (10 random individuals) to each pot, based on assigned treatment. The setting and density of species in each pot were in agreement with my field investigations (unpublished data). Plant traits were measured initially and then biweekly, including leaf count, node count, branch count, chlorophyll concentration index (CHL) (by chlorophyll meter, SPAD-502Plus), internode length, and flower count. Aphid populations were recorded weekly.

In the 7th week, this study harvested above- and below-ground materials in each pot, including above-ground plant biomass, 5 to 6 plant leaves (per pot), soil, litter remaining, and earthworms. Aboveground plant biomass was oven-dried under 70℃ for 3 days and weighted. Plant leaves were scanned to calculate leaf area (estimated by Photoshop) and then oven-dried (40 ℃) for 2 weeks. Their dry mass and SLA (specific leaf area, mass per area) were measured. Finally, these leaves were ground into fine powder and analyzed for their carbon (C) content, nitrogen (N) content, C/N ratio, and iWUE (internal water usage efficiency, calculated from C^{13}/C^{12} discrimination (Warren *et al.* 2001)). Stable isotope analysis was conducted with GC-MS (Gas Chromatography-Mass Spectrometry) (Thermo DELTA5, Technology Commons, College of Life Science, National Taiwan University). Two soil samples from each pot were preserved in plastic containers and air-dried for the analysis in soil physical and chemical properties (details in the section below). All survived earthworms were excavated and weighted. Litter remaining was collected as much as possible from the surface of each pot, rinsed to wash off soil, and dried under 60℃ for 3 days. The reduction in litter weight (10 grams minus the final weight) was calculated to approximate the litter decomposition rate in each treatment.

Soil property analysis

Four analyses were conducted for soil physical and chemical properties, including water stable aggregate distribution, soil available phosphate, nitrate concentration, and soil organic carbon.

Soil samples were sieved with 2 mm mesh or 0.25 mm mesh and analyzed by the Bray No. 1 method for soil available phosphate or by the Walkley-Black wet oxidation method for organic carbon (Martin 1993, Drechsel *et al.* 1996), respectively. Nitrate concentration was extracted by 0.01 M calcium sulfate dihydrate and measured by LAQUA Twin nitrate meter. Water stable aggregate distribution indicates how much proportion of soil aggregate remains undestroyed in different size fractions under up-down stroke of water force (simulating stormy rain) (Martin 1993). Five size fractions were used in this study: 10.0 ~ 4.76 mm, 4.76 ~ 2.00 mm, 2.00 ~ 1.00 mm, 1.00 ~ 0.50 mm, and 0.50 ~ 0.25 mm. Forty grams of 10.0 ~ 4.76 mm soil aggregates were put on the top of nested standard sieves and placed in wet sieving apparatus. I wetted the soil for 10 minutes and then activated the wet sieving apparatus for 10 minutes. The remains on each sieve were collected, oven-dried under 105℃ overnight, and weighed. Since the primary particles within the remains would contribute errors, all remains were mixed with 0.5% w/v sodium hexametaphosphate solution (as dispersion, can destroy all soil aggregates), shaken for 45 minutes, and passed the sieve again. All remains on sieves were weighed as primary particle. After adjustment for the weight of primary particles, MWD (mean weight diameter) was calculated (Appendix 1, Equation 3).

Data Analysis

This study applied multivariate ordination technique (e.g. detrended correspondence analysis, DCA and redundancy analysis, RDA), Bayesian hierarchical model, confirmatory path analysis (e.g. Shipley's directional separation), and mediation analysis (via potential outcome approach) to qualify or quantify 1) the responses of plant traits to warming, aphid, and earthworm treatments, 2) the direct effects and 3) the indirect effects of treatments on traits in this above-below-ground system, and 4) the effect size of direct and indirect effects. All analyses were done by R 3.01 (package "vegan", "MCMCglmm", "mediation", and "R2WinBUGs") and WinBUGs 14.3 (Bayesian inference using Gibbs sampling for Windows) (Michael 2007, Borcard *et al.* 2013, R Development Core Team 2013).

(1) The responses of plant traits to treatments

This study applied constrained ordination technique to examine possible relationships between plant traits and experimental factors, including treatments (earthworms, aphids, warming), experimental round (1^{st} or 2^{nd} round, treated as a random effect), or collecting site (treated as random effect). This analysis also calculated the relative effect size of treatment factors, correlations among plant traits, and potential clusters among samples. The trait values (including leaf, flower, node, branch count, internode length, and CHL) of the 2^{nd} week were subtracted from those of the 6^{th} week for evaluating the treatment effects on plant growth or development. These traits were combined with traits values at the final harvesting (including leaf C, N content, C/N ratio, C^{13}/C^{12} discrimination, above-ground biomass, number of flower, and SLA) to represent the overall response of plant. The number of nodes and leaf C/N ratio were removed because of its highly correlation with above-ground biomass (0.83) and N content (-0.97), respectively. Each value in the final matrix including 96 samples each for 10 traits were z-score transformed (subtracting mean and then dividing by standard deviation) prior to analysis. I used detrended correspondence analysis (DCA) at first for evaluating the relevant response model for how relationship between traits and samples responding to environmental factors, by examining the length of longest axis. If the length of longest DCA axis is shorter than 2, a linear model will be optimum (should apply RDA); if the length is longer than 4, a unimodal model will be optimum (should apply CCA) (Legendre & Gallagher, 2001).

Based on the result from DCA (Table 1) (longest axis length = 0.3476), the optima response approximated to a linear model, and redundancy analysis (RDA) was applied.

Forward stepwise selection procedure and permutation tests were applied for selecting important factors from all potential factors (Borcard *et al.*, 2013). This procedure selected factors one by one into an explanatory model based on the smallest deviance and Akaike Information Criterion (AIC), which accounted for both the goodness of fit in response models and penalty from the complexity in explanatory models. After the best explanatory model was selected, significant random effects within this model were partitioned out. Finally, RDA triplot and the variance explained by each factors were presented. (Figures 2, 3, and Table 2)

(2) The direct effects of treatments

The direct effects of warming, earthworm, and aphid treatments on each plant traits were analyzed by Bayesian hierarchical model (BHM). Bayesian statistics was based on posterior inference which was the joint probability distribution of prior information and maximum likelihood estimates. The posterior estimates were acquired via MCMC (Markov Chain Monte Carlo) technique and sampled by Gibbs sampler under WinBUGs software. The posterior inference was made from including massive possible values of each parameter in its sample space, and thus the 95% credible interval of effect size was acquired (Michael 2007, Ioannis 2009). Besides, for data set containing a clustered (or dependent) structure (e.g.: replicates within each chamber or repeated measures for the same replicate), BHM can borrow the prior information of between levels to make enhanced clearer inference for within a level. However, the chamber level variance was dropped in each model for decreasing an unreasonably big variance of chamber level effects (i.e.: warming and experimental round). Temporal effects were set as 2^{nd} level in BHM to compare the results at each time point (random slope and intercept model) and incorporated with auto-regressive (AR(1)) structure. Three chains and uninformative conjugate priors were used for each model. Counting data were fit with Poisson or negative binomial distribution. Diagnostic of convergence was conducted by visual check and R-hat statistics. Model quality was checked by posterior predicted check plot and posterior predicted P value (PP *P* value) (discrepancy between observed and simulated data). Comparative model fit was checked based on deviance information criterion (DIC) (WinBUGs code for the model structure: Appendix 3).

To make clearer inference, this study calculated modified Paine's index in Bayesian models. This index was defined as the change in trait value under treatment divided by the trait value under control. In short, the index represents a proportional change in trait value due to a specific treatment. (Appendix 1, Equation 1)

(3) The indirect effects of treatments

This study identified indirect effects based on the mediation analysis (MacKinnon *et al.* 2007). An indirect effect (also called mediation effect) exists when one variable can affect other variables through mediators. Thus, identification of indirect effects in a pathway requires an understanding of underlying mechanisms. For example, earthworm effects on aboveground biomass could be affected by an increase in decomposition rate, and thus change in decomposition (an underlying mechanism) could be a mediator between earthworms and aboveground biomass.

This study adopted Shipley's directional separation (d-sep) test (Shipley 2009, Clough 2012) to verify the path structure that may include indirect effects (i.e. effects created by mediators). To identify a mediator based on the d-sep test, this study first explored each potential variable that could mediate the interaction between an independent variable (IV) and a dependent variable (DV). Besides being biologically reasonable (supported by underlying mechanisms), the mediators (variables) need to be significantly affected by IV and significantly affect DV. Once these potential mediators were identified, this study would further verify the mediators by constructing relevant causal directed acyclic graphs (DAGs) and d-sep, logically testing whether the independence claims based on different causal DAG would hold or not with C-statistics (Appendix1, Equation 2). The value of C was fit to chi-square distribution with 2k degrees of freedom (k: number of claims). The causal DAG would be rejected if the P value was lower than a threshold (e.g., $P < 0.05$).

(4) The effect size of direct and indirect effects

The effect sizes of direct, indirect, or total effects of IV on DV were assessed by potential outcome approach (Imai *et al*, 2010 a, b). Average causal mediation effect (ACME), average direct effect (ADE), total effect, and proportion mediated were calculated by simulating the difference between potential outcomes under sequential ignorability assumption (Appendix 1, Equation 4). This assumption holds only when there is little correlation with pre-treatment confounding effects; otherwise the estimates of mediation analysis will become biased. Thus, this study incorporated all pertinent covariates in models and performed a sensitivity analysis to check the bias in estimates under different levels of correlations with pre-treatment confounders. The analysis of potential outcome approach would become robust once the estimates remained unchanged even under a strong violation of sequential ignorability.

This study tested three potential mediating effects in the above-below-ground system: (1) Plant traits may mediate the effects of warming or earthworm treatment on aphid population, (2) soil properties may mediate the effects of warming or earthworm treatment on plant traits, and (3) litter decomposition may mediate the effects of warming or earthworm treatment on plant traits.

Results

<u>The response of plant traits to warming, aphid, and earthworm treatments</u>

RDA analysis indicated that a combination of 10 plant traits (number of leaf, branch, flower, internode length, chlorophyll concentration, specific leaf area, aboveground plant biomass, leaf C, N content, and C^{13}/C^{12} discrimination) was well explained by 7 factors (3 treatments and 4 other experimental factors) under the forward stepwise selection procedure (Table 2). These factors included warming, aphid, earthworm, experimental round, site T (samples from site T), and site W (samples from site W). AIC reduced from 222.0 (no factor included) to 176.6 (7 factors included). Besides, a permutation test showed that including each of the factors can significantly explain the response (all P-value < 0.01). Neither two- nor three-way interactions among fixed effects (warming, earthworm, and aphid) were selected in this procedure, suggesting that these three fixed effects may independently affect plant traits.

The total variance under RDA analysis was divided to constrained (variance that can be explained by explanatory model) and unconstrained fractions. The constrained axis (RDA axis) 1 and 2 explained 32.21% variation among the 10 traits after partitioning out random effects including experimental round, site T, and site W. The position of sample points matched well with the treatment factor axis (Figure 2). For example, most samples under warming treatment followed the 'Warm' axis. Similar patterns were found for the other two treatments (axes). Sample points from control treatment and 'E+A' treatment were generally located in the middle part of RDA triplot. 'A' and 'E' axes nearly followed a straight line, with similar length, but different direction. This could suggest the aphid and earthworm effects on plant traits were in general counter to each other and with similar strength. Given that the axis 'Warm' was in general perpendicular to 'A' and 'E' axes, the effect of warming seemed to affect plant traits in a way independent from that of aphid or earthworm treatment, such as impacting different plant traits with different direction and magnitude.

Variation partitioning of the treatment effects in RDA analysis showed a big difference in the contribution of each treatment effect (Figure 3). Aphid treatment accounted for 27.0% (=8.64 / 31.96) of the total variance explained (Adjusted R-square = 31.96%), earthworm treatment accounted for 27.0% (=8.63 / 31.96), while warming accounted for 43.9% (=14.02 / 31.96) of total variance explained. All two-way interactions reduced the variance explained (W*A: -0.25%, A*E: -0.19%, and W*E: -0.25%). The three-way interaction only accounted for 0.01% of the total variance. Since incorporating interactions decreased the adjusted R-square, interaction terms were not selected by stepwise procedure.

<u>Qualify and quantify the direct effects of treatments</u>

The magnitude (i.e. effect size) of warming, earthworm, and aphid impacts on plants were similar on five plant traits that had been repeated measured (e.g. leaf, node, branch, mean internode length, and chlorophyll concentration index) (Figure 4). In particular, the effect sizes of the 5 traits were presented as Paine's indices, and it represented the proportional change of that trait under treatments. For example, Paine's index for leaf counts under warming in week 2 roughly equaled to + 0.8 (Fig. 4A), indicating that warming increased the leaf count by 80 %, compared to control. The analysis in effect size (Fig 4) revealed three general patterns. First, warming had relatively stronger positive impacts on the 5 plant traits in this system, but the impacts decreased linearly as time progressed (Fig 4A-E). Second, the effect sizes of earthworm and aphid treatments were similar but differed in direction (Fig 4A-E), consistent with the result of the RDA analysis (Fig 2). Third, the effects of earthworm and aphid treatments on most plant traits were relatively small or undetectable (non-significant) by week 4 but became significant and stronger in week 6, although the earthworm effects on branch and node count were similar between week 4 and week 6. .

Plant traits collected by destructive sampling methods were shown in Table 3. Aphid treatment increased SLA by 38.180 cm^2/g (95% CI: 26.770 ~ 49.530 cm^2/g). Warming and earthworm treatment increased aboveground biomass by 1.057 and 0.543 g respectively (95% CI: 0.7357 ~ 1.378 g for warming, 0.222 ~ 0.864 g for earthworm), and aphid treatment decreased aboveground biomass by -0.767 g (95% CI: -1.086 ~ -0.447 g). The effect size on aboveground biomass was warming (+) ≥ earthworm (+) > aphids (-). As for internal water use efficiency in plants, only warming treatment had a significantly negative effect on it (-2.168, 95% CI: -3.979 ~ -0.360). All treatments significantly increased nitrogen content (95% CI: 0.105 ~ 0.269 % for warming, 0.144 ~

0.308 % for aphid, and 0.143 ~ 0.308 % for earthworm).

Treatments showed some direct effects on aphids and earthworms as well. Warming negatively affected aphid populations in week 3 and 4 (95% CI: -0.781 ~ -0.365 at week 3 and -0.724 ~ -0.192 at week 4), while aphid populations generally performed well in growth chambers and increased log-linearly (or exponentially) from week 2 to 7 (Figure 5). Aphid population size tended to benefit from earthworm treatment in week 6 and 7 (90 % CI: 0.007 ~ 0.451 at week 6 and 0.006 ~ 0.451 at week 7, Figure 6). Earthworm survival was not affected by aphid and warming treatments but earthworm body weight (e.g. change in weight during the experiment) was negatively affected by warming (95% CI: -0.413 ~ -0.099).

Treatments affected some soil physical properties but not soil chemical property during the study period. The larger size fraction of water stable aggregates responded to earthworm, aphid, or warming treatment (Table 4). Earthworm treatment had a positive effect on size fraction 10.0 ~ 4.76 mm (95% CI: 0.652 ~ 2.210) and 4.76 ~ 2.00 mm (95% CI: 0.079 ~ 0.529), and a marginally positive effect on size fraction 2.00 ~ 1.00 mm (90% CI: 0.068 ~ 1.082). Aphid treatment had a marginally negative effect on size fraction 4.76 ~ 2.00 mm (90% CI: -0.393 ~ -0.019). Warming had a positive effect on size fraction 4.76 ~ 2.00 mm (95%CI: 0.064 ~ 0.515). For the summation index of water stable aggregate distribution, MWD, only earthworm treatment had an effect (positive) on it (95% CI: 0.465 ~ 1.088). No effects were found on soil chemical traits (i.e. available phosphate and organic carbon). This study also measured soil nitrate, but the concentration was too low to be detected by our machine.

The amount of litter decomposition was higher in warming and earthworm treatments (Figure 7). In other words, warming and earthworm treatments increased litter decomposition (95% CI 1.217 ~ 1.875 g for earthworm; 0.520 ~ 1.176 g for warming).

Qualify and quantify the indirect effects of treatments

(1) Indirect effects of warming and earthworm treatments on aphid population – plant traits as mediators

A marginally significant effect of earthworm treatment on aphid population in week 7 was found (90% CI: 0.006 ~ 0.451, Figure 6). Since earthworms were less likely to affect aphid population directly, this study examined whether earthworms indirectly affected aphids via a mediator (i.e. plants). Two plant traits, number of branch and N content, were mediator candidates because the two traits could be significantly affected by earthworms and significantly affect aphid population (Appendix 2, Table S1). To verify the pathway of these indirect effects and mediators, this study constructed and examined 3 possible DAGs (Figure 8): (1) E → Branch → ln(Aphid), E → N content→ ln(Aphid); (2) E → N content → Branch → ln(Aphid); and (3) E → Branch → N content → ln(Aphid). The independence claims of each DAG were listed and tested by C statistics (Table 5). DAG1 fitted best (P value = 0.215) and thus indicated that the indirect effect of earthworm treatment on aphid population in week 7 was separately mediated by number of branch and N content. However, significant ACME was only found for N content mediating earthworm effect on aphid (mean: 0.164, 95% CI: 0.001 ~ 0.389), and the proportion of indirect effect was 0.488 (90 % CI: 0.061 ~ 1.421, marginally significant) (Appendix 2, Table S2). Detailed standardized estimates of coefficients were shown in Figure 9. These coefficients represented the relative magnitude of direct effects in this DAG. Note that the direct effects of earthworm treatment on branch number and nitrogen content (mediators) were stronger than the direct effects of mediators on aphid population.

This study also examined the possibility that warming may indirectly affect aphid population through a mediator (e.g. plant traits), since warming treatment significantly affected both aphid population and plant trait. For example, this study showed that 5 plant morphological traits (leaf, node, branch, chlorophyll, and internode length) in week 2 could actually affect aphid population in week 3 and be affected by warming. Owing to high correlations among these 5 plant traits, PC 1 (first axis from principle component analysis) which accounted for 89.2 % variance of the 5 traits was fitted in mediation analysis. The result indicated that there was insignificant ACME (95% CI: -0.597 ~ 0.348), significant ADE (95% CI: -0.669 ~ -0.023), and significant total effect (95% CI: -0.868 ~ -0.689) (Appendix 2, Table S2). Furthermore, it was also likely that aphid mediated warming effects on PC 1 of plant traits. The mediation analysis showed the similar results that ADE (95% CI: -3.853 ~ -1.854) and total effect (95% CI: -3.794 ~ -1.854) were significant while ACME (95% CI: -0.564 ~ 0.333) was not (Appendix 2, Table S2). Besides, sensitivity analysis showed that both inferences about indirect effect (warming → plant → aphid, or warming → aphid → plant) only hold under relative small correlation with pretreatment confounder effects (rho: -0.2 ~ 0.3 and -0.3 ~ 0.4). This indicated both inferences were not robust.

(2) Indirect effects of warming and earthworm treatments on plant traits – soil

properties as mediators

Earthworm and warming impacts on plants were likely to be mediated by increase in soil properties. This analysis included four plant traits: the change in leaf and node number from week 2 to 6, aboveground biomass, and N content because of these following reasons. The change in leaf number was significantly affected by earthworm treatment. The change in node number and aboveground biomass were significantly affected by WSA 2.00 ~ 4.76 mm, earthworm, and warming treatments. Plant nitrogen content was significantly affected by WSA 10.0 ~ 4.76 mm and earthworm treatment. The treatments, mediators, and confounders for each plant trait used for mediation analysis were listed in Table 6. After considering relevant confounding effects in model, this study found that WSA 2.00 ~ 4.76 was the only mediator significantly mediating the effects of warming and earthworm treatments on one of four focal plant traits (i.e. the change in plant node number) (95% CI of ACME: 0.469 ~ 5.865; proportion mediated: 21.1% (95% CI: 0.8% ~ 70.9%) for earthworm effect, 15.7% (95% CI: 1.3% ~ 38.9%) for warming effect) (Appendix 2, Table S2). This study did not detect other mediators for the other three plant traits (change in leaf number, aboveground biomass, N content; Table 6), suggesting that those 3 plant traits were directly affected by warming or earthworm treatment (Table 6). The DAG and standardized coefficients were shown in Figure 10. The structural fit for this DAG was supported by Bayesian framework with the PP p-value at 0.51 (not far from 0.5), which indicated that this model was plausible (Lee 2007). Although WSA 2.00 ~ 4.76 was a mediator, the direct effect of WSA 2.00 ~ 4.76 on growth of node number was relatively weak compared to other direct treatment effects in this DAG (Fig. 10)

Discussion

1. The non-contingent response of above-below-ground interactions to warming scenario

This study showed that the above-below-ground interactions were similar under normal and warming scenarios, suggesting a non-contingent response to warming. Although warming, aphid, and earthworm effects were found on several plant traits, there was no significant interactions between warming and other treatment effects. This was supported by a number of data analyses:(1) no interactions was detected during the forward stepwise selection (Table 2), (2) the perpendicular axes between warming and aphid-earthworm axes in RDA triplot (Figure 2), (3) including interaction terms reduced the total variance explained (Figure 3), (4) an increase of DIC by 3 for adding each interaction in effect size estimation, and (5) non-significant effect size of interaction term. All analyses above indicated that the interactions among warming, aphids, and earthworms didn't exist in this study.

Although this study did not detect any interaction in the impact of treatments (warming, aphids, and earthworms) on plant traits, this study found that both earthworms and aphids were actually affected by warming. Given that the performance of earthworms and aphids did change under warming, how could they maintain the similar impacts on plants (based on effect size) under normal and warming scenarios? The possible mechanisms were discussed as below:

(1) Warming had a negative effect on aphid population size at the beginning of aphid colonization, but this effect disappeared after 2 weeks. Two reasons could explain why warming did not indirectly affect plants by decreasing aphid population size at the beginning of aphid colonization. First, this study did not detect aphid effect on plant traits in the first 2 weeks of aphid colonization, and this coincided with relatively small sizes of aphid populations. Namely, the effect of aphids on plants could be population size dependent (Underwood, 2000). In this study, warming's

negative effect on aphids happened exactly when aphid populations were low and had little effect on plants. Hence, warming affected aphids but that effect was not strong enough to impact plants. Second, mediation analysis indicated that the decreased aphid population size by warming only ramified insignificant weak indirect effect. Thus, warming didn't produce much indirect effect on top of its direct effect on aphids.

(2) Earthworms under warming treatment lost more weight, but their impact on plant traits were similar between control and warming treatments. In general, earthworm can affect plants through 2 pathways, decomposition and ecosystem engineering. The former one may result in increased soil nutrients and the latter one may modify the soil structure. In my study system, both pathways (litter decomposition and WSA 2.00 ~ 4.76 mm) benefited from warming. Therefore, it is possible that the positive combined effects of earthworms and warming on decomposition and ecosystem engineering could offset the negative effect of decreased earthworm performance on plant traits under warming. As a result, this study found similar earthworm impact on plants under control and warming treatments. Furthermore, there was actually little evidence that losing more body weight in earthworms due to

warming would lead to a decrease in earthworm activities and subsequent benefits for plants. Indeed, Zaller *et al.* (2009) provided an example where earthworm's effect on plants remained similar although warming significantly decreased earthworm's density and biomass. I argue that the amount of weight loss and temporal scale might play an important role in the lack of association between earthworm biomass and plant performance in this study. First, it is possible the weight loss in earthworms under warming had not reached a threshold, and thus the positive effect of earthworms on plants had not decreased yet. Second, the temporal scale required to observe the negative effect of reduced earthworm biomass on plants may be longer than expected. Assuming that earthworm effects on plants accumulates with time, then extending study period may increase the possibility to observe a difference in earthworm effect between control and warming treatments. Besides, given that the effects of ecosystem engineering can last longer than the life time of ecosystem engineers (Hastings *et al.* 2007). The ecosystem engineering effects of earthworms on plants may last even when warming treatment directly reduces earthworm biomass.

2. The independence between above- and below- ground effects on plants

Besides being independent of warming treatment, the aphid (above-ground) and earthworm (below-ground) effects on plants were also independent of each other. In other words, the effects of earthworms on plants under aphid or aphid-free treatment were the same, and vice versa. This is intriguing because this study found that earthworm treatment marginally increased aphid populations in week 6 and 7 (figure 6), while aphid treatment didn't affect the earthworms (survivorship and body weight). In specific, after back-transformation (exponential), the difference in average aphid populations between earthworm and earthworm-free treatments was about 284 and 514 individuals in week 6 and 7, respectively. So why did this study detect no interaction between earthworm and aphid treatments (i.e. higher aphid populations under earthworm treatments should have caused more damage on plants)? The positive earthworm impact on aphid population was also observed by Wurst *et al.* (2003) and Poveda *et al.* (2005), although they did not explicitly examine the interaction between aphid and earthworm effect on plant performance. The lack of interaction may be explained by the variation in effect size through time in this study, where the effect size of a) aphid and earthworm effects on plant traits and b) earthworm effect on aphid populations both increased with time. Both effects were negligible and weak at week 4 but become significant and increased in effect size at week 6 (Figure 4, 5). Extending study period may increase the possibility to observe the interaction (under additive) between earthworm and aphid effects on plants. For example, it is possible that damages from higher aphid population due to earthworm treatment will eventually cause more damage on plants. On the other hand, earthworm effect on plants will not be affected by aphid treatment and keep its positive effect on plants. Thus, I may detect an under-additive interaction between aphid and earthworm effects on plants. Another possible explanation was that the negative aphid effect on plant may have saturated during the study period. Therefore, no matter how much aphid population increased, as long as over a threshold, aphid damage on plant would remain similar. This damage saturation could be caused by intra-specific competition of aphids. This saturation, if true, could highlight a need to assess the relationship between aphid population and their damage on plants, since different relationships may lead to very different implications. For example, a linear relationship will suggest that higher aphid population will result in more damage on plants, until the system collapse. The under-additive interaction between aphids and earthworms on plants should be observed in linear relationship. A logistic relationship may imply that aphid damage is negligible below a threshold of aphid population, and saturates after certain aphid population size. The logistic relationship could explain why an increase in aphid population under earthworm treatment did cause more plant damage in this study.

This study found a one-way relationship between the above-ground (aphids) and below-ground (earthworms) components: earthworms benefited aphids, but aphids had no effect on earthworms. In theory, aphids could affect earthworms by changing litter quality and quantity. In this study, aphids significantly increased SLA and could potentially change the litter quality of plants for earthworms. However, accumulating relevant amount of litter, which was affected by aphids and then posed a strong impact on earthworms, could be difficult, because it would take lots of time for *C. diffusa* to produce this amount of litter but aphid population may not sustain for a long time in the laboratory or field (due to limited plant resource or natural predators).

3. *Mediators in above-below-ground interactions*

This study highlighted 2 important mediators in the above-below-ground system: plant nitrogen content and soil physical properties. In a biological system, a direct effect could ramify several indirect effects through mediators. In above-below-ground system, plants were generally viewed as key mediators, but relatively little was considered about soil quality as mediator. Soil quality can actually play an important role as a mediator. For example, decomposers may help release litter nutrient to soil and then indirectly affect plants. Furthermore, ecosystem engineers in soil (e.g. earthworms) could increase soil nutrient and water availability by changing soil physical properties (Crooks 2002, Lavelle *et al.* 1997). As a result, soil chemical and physical properties could serve as important mediators in above-below-ground interactions.

The mediation analysis showed that leaf nitrogen content mediated earthworm effect on aphid population. This result was consistent with those in several studies (Wurst *et al.* 2004, 2010), which suggested that nitrogen content was a key mediator of the positive or negative earthworm effect on aphid population. However, their studies did not explicitly examine the mediating mechanisms, and therefore their results could be a co-response to earthworm treatment. If the true mechanism is that earthworms affect plant nitrogen content but affect aphid population through other ways (not via plant nitrogen), the same conclusion, without conducting mediation analysis, will still be made, although it might provide misleading mechanisms. My study applied mediation analysis and explicitly tested whether increase of nitrogen was co-response or it was a key mediator by potential outcome approach. This advanced technique suggested that nitrogen content was actually a significant mediator, and the mediating effect from earthworms was 0.001 ~ 0.389. About 48.8 % of the total effect of earthworms on aphids was mediated by nitrogen, while the rest was from unmeasured mediators. Besides, branch number could affect aphid population and be affected by earthworms significantly, but potential outcome approach explicitly indicated that this could be just a co-response to earthworm treatment.

The soil chemical, physical properties, and litter decomposition were used to examine how they mediated earthworm effects on plants. The soil physical properties (WSA 10.0 ~ 4.76 and 4.76 ~ 2.00 mm) and litter decomposition were actually significantly affected by earthworms. However, mediation analysis suggested that litter decomposition and WSA > 4.76 mm (insignificant) were just a co-response to earthworm treatment, while WSA 4.76 ~ 2.00 mm was actually a significant mediator (proportional mediated = 21.1%). About 80% of earthworm effects on plants were via unmeasured mediators. In fact, a non-significant effect does not necessarily indicate that it is not an underlying mechanism. For example, if the soil is very infertile and any nutrients released from litter are immediately used up by plants, soil nutrients can be a mechanistic explanation for better plant performance but this mechanism may not be detected by comparing the change in soil nutrients. In this study, it’s possible that the insignificant soil available phosphate, organic carbon, or even non-extractable nitrate concentration were among the underlying mechanisms for earthworm effect on plant, but due to poor soil quality, every released nutrients were absorbed by plants immediately.

The earthworm *Amynthas corticis* had a strong potential for supporting pedogenic process through bioturbation (Garcia & Fragoso 2002) and placing a strong effect on water stable soil macro-aggregates (WSA > 2.00 mm) (Snyder *et al.* 2009). Moreover, an increase in WSA > 2.00 mm is generally positively correlated with organic carbon and nitrogen content (Andruschkewitsch *et al.* 2013; Adesodun *et al.* 2005). The similar pattern was also found in this study where *Amynthas corticis* had a very big impact on WSA > 2.00 mm and could mediate a small portion of earthworm effects on plants. The unmeasured mediation effect could come from the associated soil properties with WSA > 2.00 mm.

4. Direct and indirect warming impacts

This study reveals that warming can affect an above-below-ground system both directly and indirectly. Direct warming effects were found on aphid population in early colonization, plant traits (including leaf, branch, node, chlorophyll concentration, internode length, above-ground biomass, internal water use efficiency, nitrogen content), soil physical property (WSA 4.76 ~ 2.00 mm), litter decomposition, and earthworm weight change. However, the effect size of warming on plant traits decreased with time in the study system.

Warming indirectly affected plants through changing soil properties in this study system. Both litter decomposition and WSA 4.76 ~ 2.00 mm were positively affected by warming, and mediation analysis showed that warming impact on plant node number (e.g. change from week 2 to 6) was mediated by WSA 4.76 ~ 2.00 mm. Few studies have shown that warming can indirectly affect plants through changing soil physical property (e.g. WSA 4.76 ~ 2.00 mm in this study), but Eisenhauter *et al.* (2011) suggested that warming could indirectly affect exotic seedlings through decreasing soil water content. Based on my pre - experiment, soil water content under warming treatment decreased from 25.89% (95% CI: 24.80% ~ 26.98%) to 14.97 % (95% CI:

13.88% ~ 16.05%) in 7 days, while that under normal scenario only decreased from 30.16 % (95% CI: 29.00% ~ 31.31%) to 23.40 % (95% CI: 22.24% ~ 24.56%) in 7 days. The decrease in disruptive force (water content) in formation of water stable aggregate could be the reason for increase in water stable aggregate in my study (Utomo 1982).

5. The problem of scale and generalization

Every pattern observed is scale dependent (Levin 1992; Chave 2013). All patterns revealed in this study were unavoidably restricted in the mosaic of temporal (about 2 months) and spatial (the size of growth chambers or pots) scale. Making global-scale predictions in above-below-ground interactions under climate warming directly based on this study could be inappropriate. However, this empirical study provides valuable insights into potential mechanistic explanations for patterns at larger scale.

For example, the change in the effect size of earthworms, aphids, and warming across time period in this small-scale experiment may suggest that temporal scale could be an important factor for making inferences at larger scale. It is arguable that the spatial scale in this study may offer limited resource to organisms (e.g. plants) and lead to a bias in effect size (e.g. plant trait response), and therefore the results from this study may not be observed in a larger scale system. However, since resource limitation is ubiquitous in systems at various scales, I argue that the warming impact on above-below-ground systems at a larger scale could decrease with time, consistent with the results of this study.

6. Conclusions

While the interactions in above-below-ground systems were generally context-dependent (e.g. depending on system fertility or temporal scale), this study suggests that these interactions can be independent of warming. Namely, warming did not interfere in the interactions among plants, earthworms, and aphids in this above-below-ground system. Furthermore, the positive direct effect of warming on plants subsided linearly and became insignificant for some plant traits at the end of experiment, while the positive effect of earthworms and negative effect of aphids on plants increased with time. Given that biological (e.g. aphids, earthworms) effects and abiotic (e.g. warming) effects on plants were time dependent, this study suggests the importance to consider temporal effects as we make inferences about warming, top-down, and bottom-up effects in above-below-ground systems.

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Table 1. Output of DCA analysis for determining relevant response model of constrained ordination. RDA was applied because the length of the longest axis (bold) was shorter than 2.

	DCA1	DCA2	DCA3	DCA4
Eigenvalues	0.006951	0.002879	0.001403	0.000484
Axis length	**0.3476**	0.2570	0.1675	0.1040

Table 2. Stepwise procedure for selecting factors to explain the ordination of plant traits based on deviance and AIC. P-values were determined by permutation test (2000 times).

Factor	Step in	Deviance	AIC	P-value
…	0	…	222.0	
Warming	1	141.769	208.5	0.0005
Aphid	2	91.195	199.0	0.0005
Earthworm	3	91.075	188.0	0.0005
Round	4	44.556	182.9	0.0005
Site T	5	42.478	177.6	0.0005
Site W	6	17.409	176.6	0.0075

Table 3. Estimates of plant traits (by destructive sampling) fitted with linear model in WinBUGs. Mean and 95% credible intervals for intercept, and effect size of warming, aphid, and earthworm were shown. Numbers in bold indicate significant effects (the 95% CI did not include 0).

Plant traits	SLA (cm^2/g)		AGB (g)	
	Mean	95% CI	Mean	95% CI
Intercept	193.200	180.500 ~ 205.900	1.924	1.566 ~ 2.282
Warming	7.663	-3.693 ~ 18.990	**1.057**	**0.7357 ~ 1.378**
Aphid	**38.180**	**26.770 ~ 49.530**	**-0.767**	**-1.086 ~ -0.447**
Earthworm	-1.005	-12.310 ~ 10.290	**0.543**	**0.222 ~ 0.864**
Plant traits	**iWUE ($\delta^{13}C$ inferred)**		**N (%)**	
	Mean	95% CI	Mean	95% CI
Intercept	63.460	61.430 ~ 65.480	1.071	1.071 ~ 1.162
Warming	**-2.168**	**-3.979 ~ -0.360**	**0.187**	**0.105 ~ 0.269**
Aphid	-0.826	-2.628 ~ 0.977	**0.226**	**0.144 ~ 0.308**
Earthworm	-0.349	-2.170 ~ 1.461	**0.226**	**0.143 ~ 0.308**

Table 4. Estimates of water stable aggregates of each size fraction and mean weight diameter fitted with linear model in WinBUGs. All data were ln-transformed prior to analyze. Mean and 95% credible intervals for intercept, and effect size of warming, aphid, and warming were shown. Numbers in bold were statistically significant (the 95% CI did not include 0). Numbers with asterisk indicated marginally significant effects (90% CI did not include 0).

Ln (WSA) (%)	> 4.76 mm	4.76~2.00 mm	2.00~1.00 mm
	Mean (95 %CI)	Mean (95 %CI)	Mean (95 %CI
Intercept	-0.514 (-1.291 ~ 0.262)	0.139 (-0.086 ~ 0.365)	-0.056 (-0.662 ~ 0.554)
Warming	0.137 (-0.642 ~ 0.916)	**0.289 (0.064 ~ 0.515)**	-0.297 (-0.906 ~ 0.309)
Aphid	-0.389 (-1.168 ~ 0.390)	-0.206 (-0.431 ~ 0.018)*	-0.241 (-0.848 ~ 0.368)
Earthworm	**1.432 (0.652 ~ 2.210)**	**0.303 (0.079 ~ 0.529)**	0.574 (-0.034 ~ 1.184)*

Ln (WSA) (%)	1.00~0.50 mm	0.50~0.25 mm	MWD
	Mean (95 %CI)	Mean (95 %CI)	Mean (95 %CI
Intercept	0.773 (0.551 ~ 0.995)	1.421 (1.150 ~ 1.690)	2.176 (1.862 ~ 2.488)
Warming	-0.12 (-0.341 ~ 0.101)	-0.172 (-0.445 ~ 0.099)	0.149 (-0.164 ~ 0.409)
Aphid	0.107 (-0.115 ~ 0.328)	0.049 (-0.222 ~ 0.333)	-0.094 (-0.405 ~ 0.218)
Earthworm	0.109 (-0.115 ~ 0.332)	0.156 (-0.117 ~ 0.427)	**0.777 (0.465 ~ 1.088)**

Table 5. D-sep test for 3 possible DAGs in figure 10. If the DAG is correct, each independence claim should hold and the null probability should be far from significant. The C statistics were used to combine the null probability in each claim and fit with chi-square distribution to gain p-value. The p-value of DAG1 was bigger than 0.05, suggesting that this DAG was supported by data. Models with asterisk were fitted with poisson model, otherwise were fitted with linear model. A7: aphid population at week 7; E: earthworm treatment; B: number of branch; N: nitrogen content.

	D-sep claim of independence	Models	Coefficient should be zero	Null probability	C statistics / P-value
DAG1	{E, A7}|{B, N}	A7 ~ E + B + N	E	0.4028	C = 5.791
	{B, N}|{E}	N ~ B + E	B	0.1372	**P = 0.215**
DAG2	{E, B}|{N}	B ~ E + N *	E	0.0658	C = 19.449
	{N, A7}|{B}	A7 ~ N + B	N	0.0090	P = 0.003
	{E, A7}|{B}	A7 ~ E + B	E	0.1010	
DAG3	{E, N}|{B}	N ~ E + B	E	0.0152	C = 14.875
	{B, A7}|{N}	A7 ~ B +N	B	0.1626	P = 0.021
	{E, A7}|{N}	A7 ~ E + B	E	0.2382	

Table 6. Summary for mediation analysis for whether earthworm and warming effects on plant traits were mediated by soil properties. The 95% confidence intervals of each ACME and ADE were shown for each model. Letters in bold indicate statistically significant. (D-leaf or D-node: difference of leaf or node count between week 6 and 2; AGB: aboveground biomass; N: nitrogen content; W: warming treatment; E: earthworm treatment; D: litter decomposition rate; WSA_2 and WSA_476: water stable aggregates with size fraction of 4.76 ~ 2.00 mm and > 4.76 mm; A: aphid treatment; R: experimental round)

Trait	Treatment	Mediators	Confounders	ACME	ADE
D-leaf	E	D	A, R, W	D: -6.328 ~ 10.779	**E: 6.118 ~ 30.147**
D-Node	W E	WSA_2	A, R,	**WSA_2: 0.786 ~ 5.427**	**W: 7.064 ~ 21.469** **E: 1.406 ~ 16.746**
AGB	W E	WSA_2	A, R,	WSA_2: -0.047 ~ 0.336	**W: 0.606 ~ 1.549** **E: 0.094 ~ 1.181**
N	E	WSA_476	W, A, R	WSA_476: -0.012 ~0.141	E: -0.005 ~ 0.304

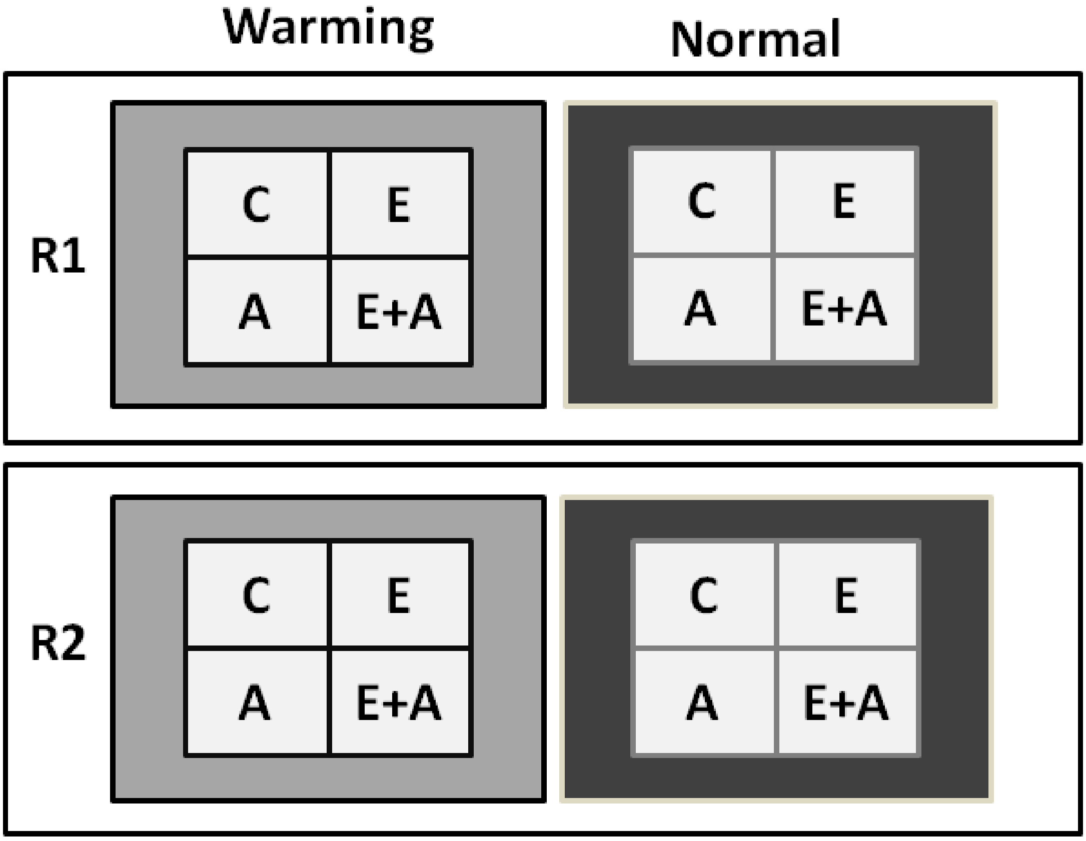


Fig. 1 Experimental layout for pots and chambers. R = experimental round, C = control, E= earthworm, A = aphid. Two growth chamber (warming and normal scenarios) were used for each round. Each growth chamber contained 24 pots (6 replicate / treatment).

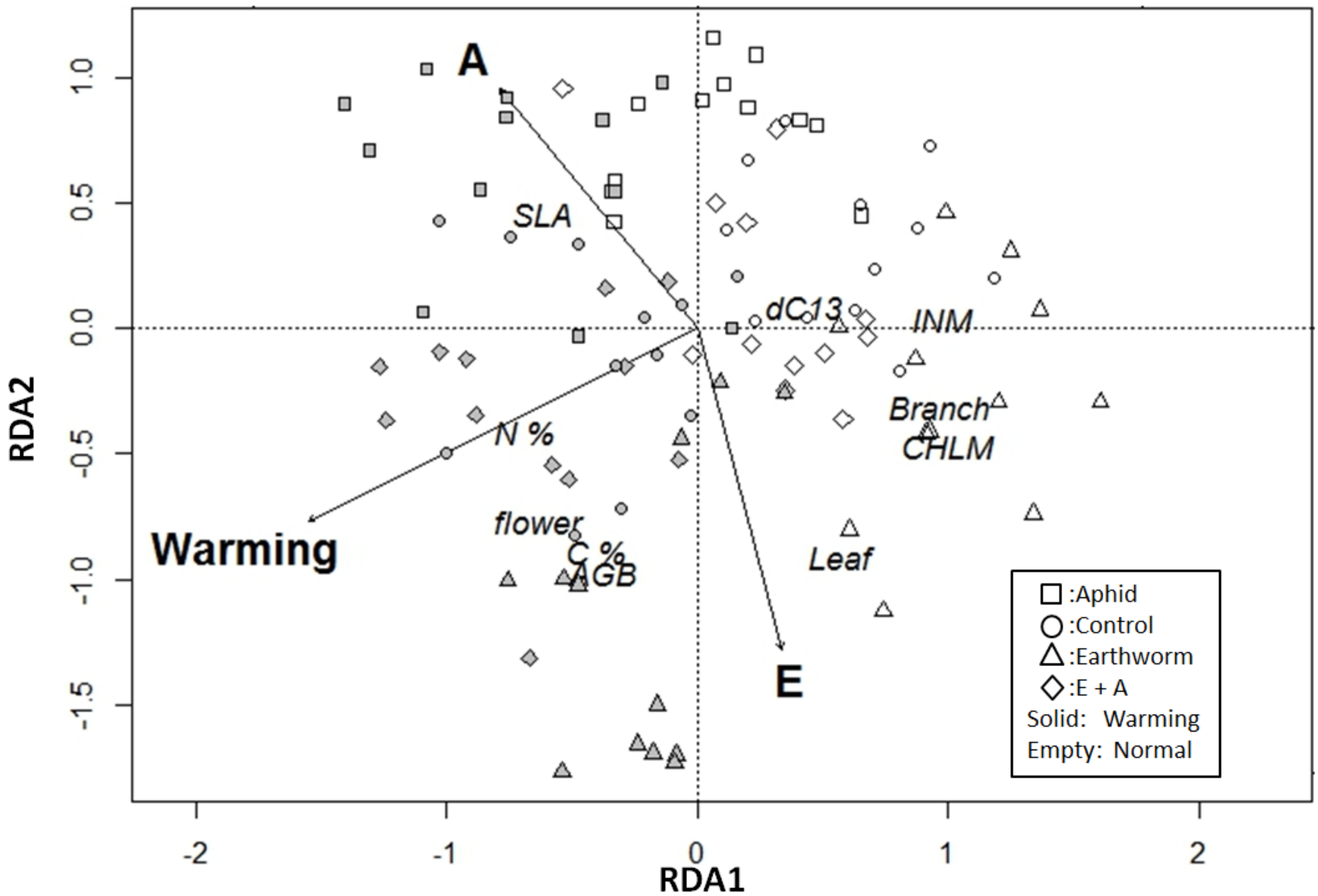


Fig. 2 Ordination triplot of RDA results for plant traits explained by treatments. Significant random effects including round, site T, and site W were partitioned out. Letters in italic are plant traits (SLA: specific leaf area, Leaf: number of leaf, flower: number of flower, INM: mean of internode length, Chl: chlorophyll concentration index, Branch: number of branch, AGB: above-ground biomass, C%: carbon content, N%: nitrogen content, and dC13: C^{13}/C^{12} discrimination). Leaf, INM, Chl, and Branch were trait value difference between week 2 and week 6. Letters in bold are treatment factors (A: aphid, E: earthworm, and Warm: warming). Solid points represent replicates from warming scenario, else are from normal scenario. Square, circle, triangle, and diamond points indicate replicates of with aphid, control, earthworm, and aphid plus earthworm treatment, respectively.

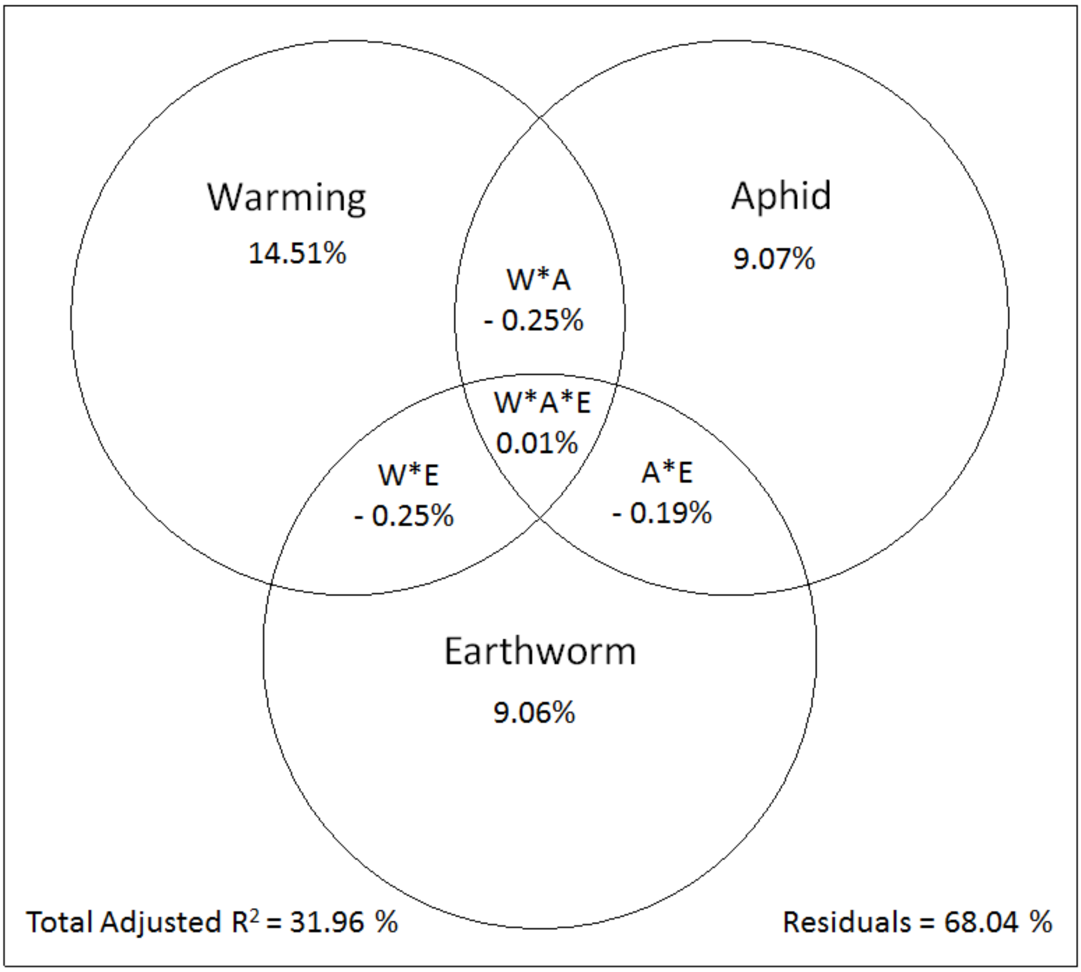


Fig. 3 Variance partitioning for plant traits by treatment factors based on RDA analysis. Total variance explained = 0.3196. Each part indicates the variance explained by an independent effect. For example: Aphid effect only = 8.64 %; Total aphid effect = aphid effect + interactions = 9.07 % - 0.25 % - 0.19 % + 0.01 % = 8.64 %.

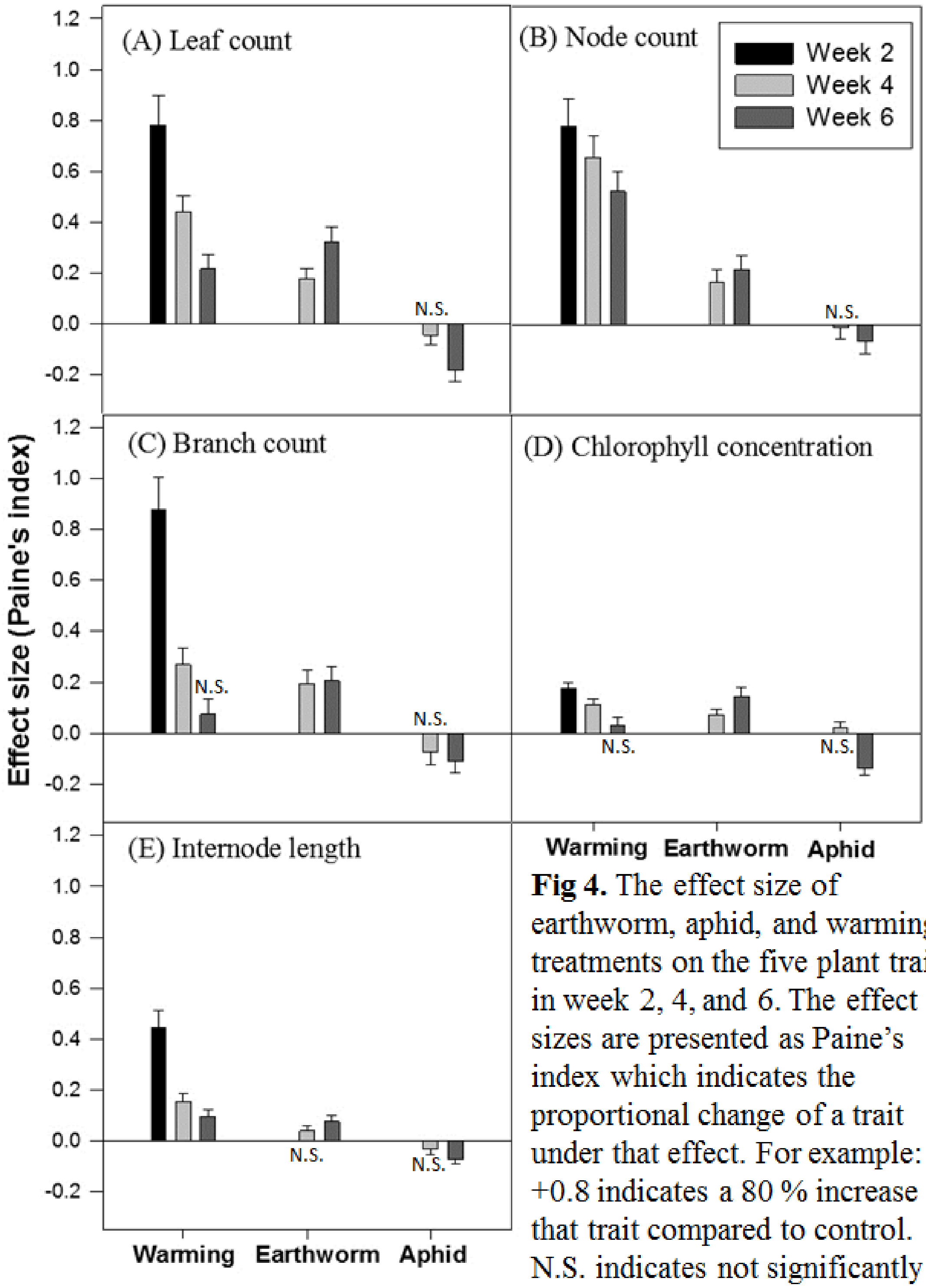


Fig 4. The effect size of earthworm, aphid, and warming treatments on the five plant traits in week 2, 4, and 6. The effect sizes are presented as Paine's index which indicates the proportional change of a trait under that effect. For example: +0.8 indicates a 80 % increase of that trait compared to control. N.S. indicates not significantly differ from 0.

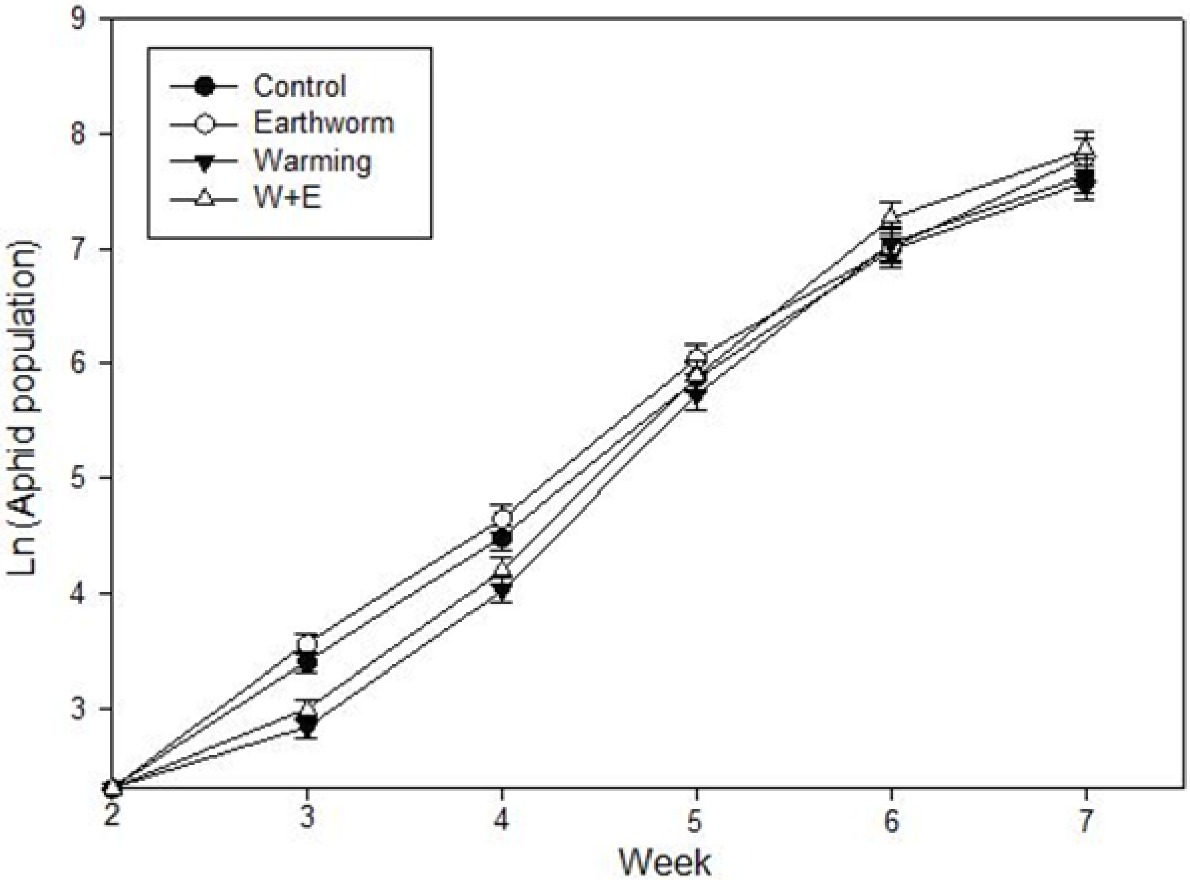


Fig. 5 ln(Aphid population) under each treatment from week 2 to 7. This figure was based on estimates from BHM which incorporating autocorrelation among weeks, rather than raw data.

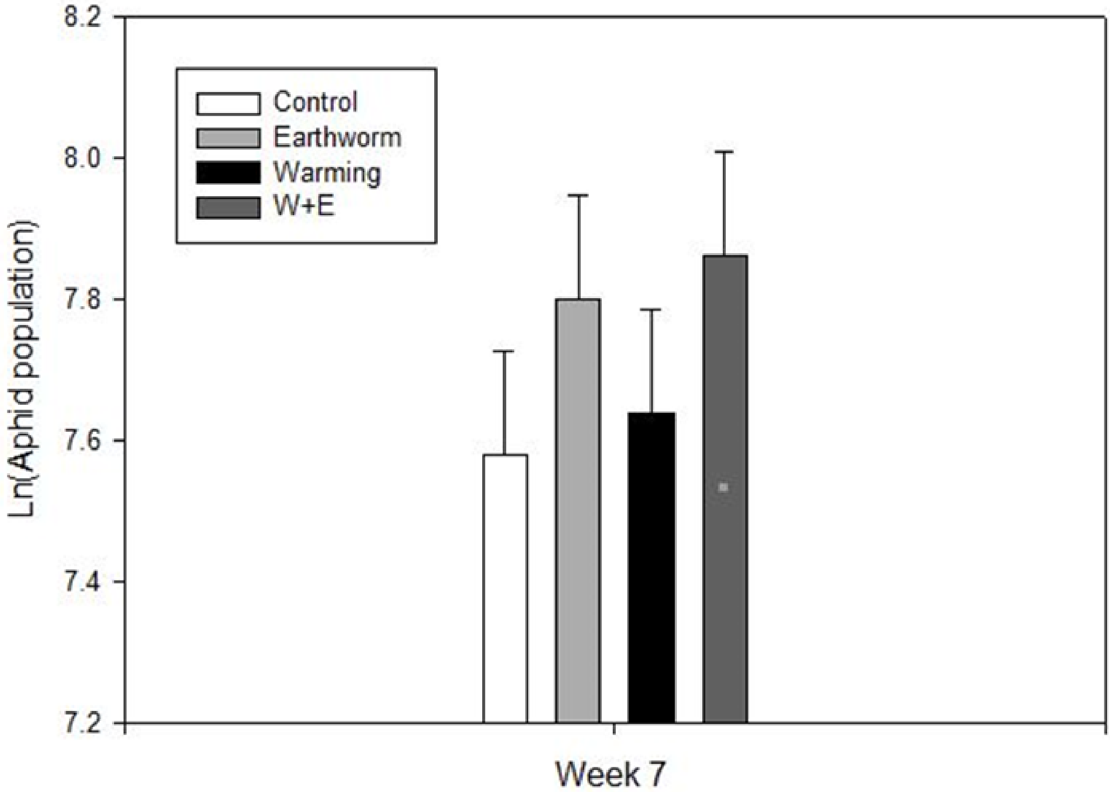


Fig. 6 ln(Aphid population) in week 7 under different treatments

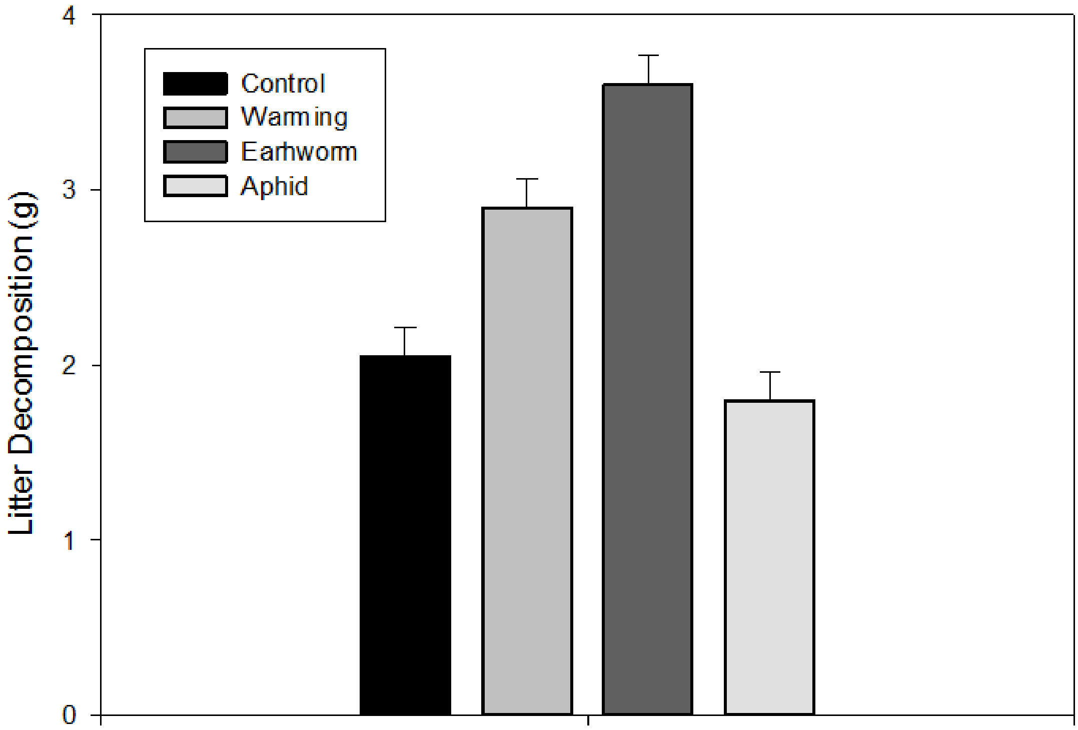
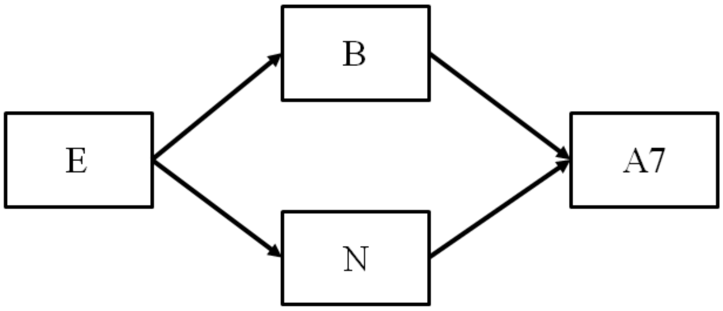
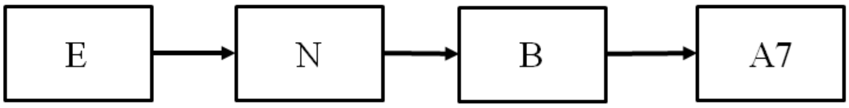


Fig. 7 Litter decomposition under different treatments

DAG 1



DAG 2



DAG 3

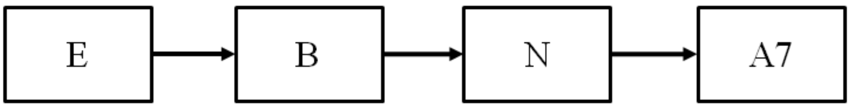


Fig. 8 The 3 possible DAGs of mediation effect of earthworms on aphid population. A7: aphid population in week 7; E: earthworm treatment; B: number of branches; N: nitrogen content.

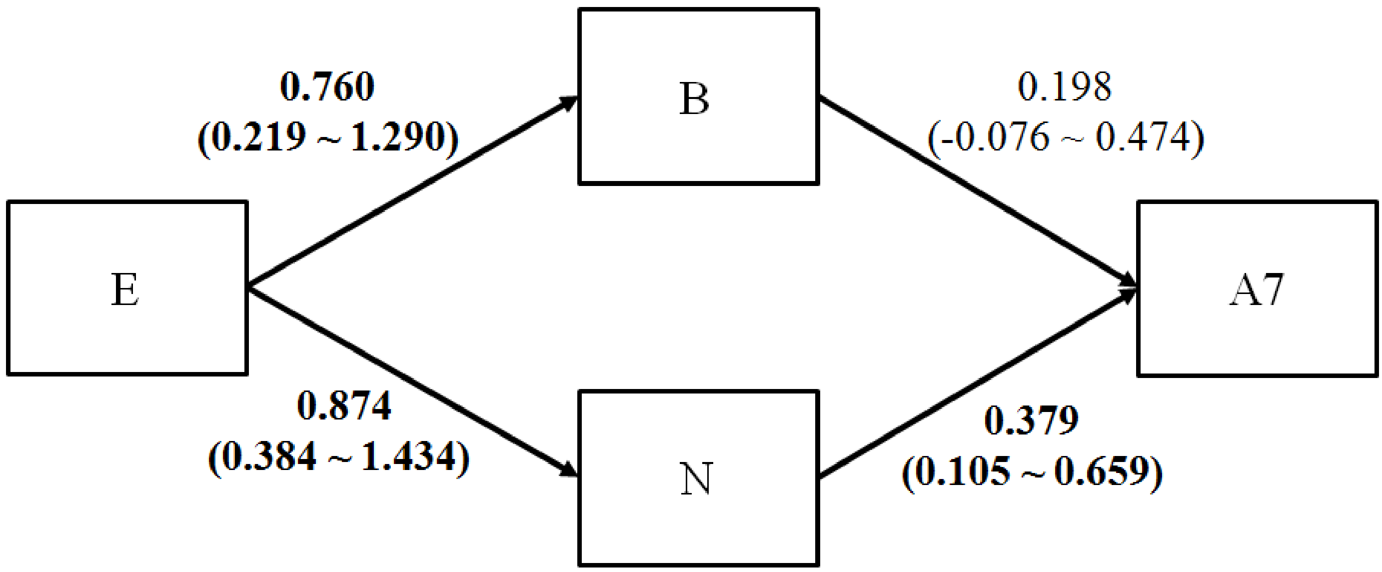


Fig. 9 DAG with standardized coefficients for how earthworms affected aphid population in week 7 via plant traits (nitrogen content and number of branch). Averaged and 95%CI of effect size for each path are shown. Letters in bold indicate significantly different from 0. A7: aphid population at week 7; E: earthworm treatment; B: number of branch; N: nitrogen content

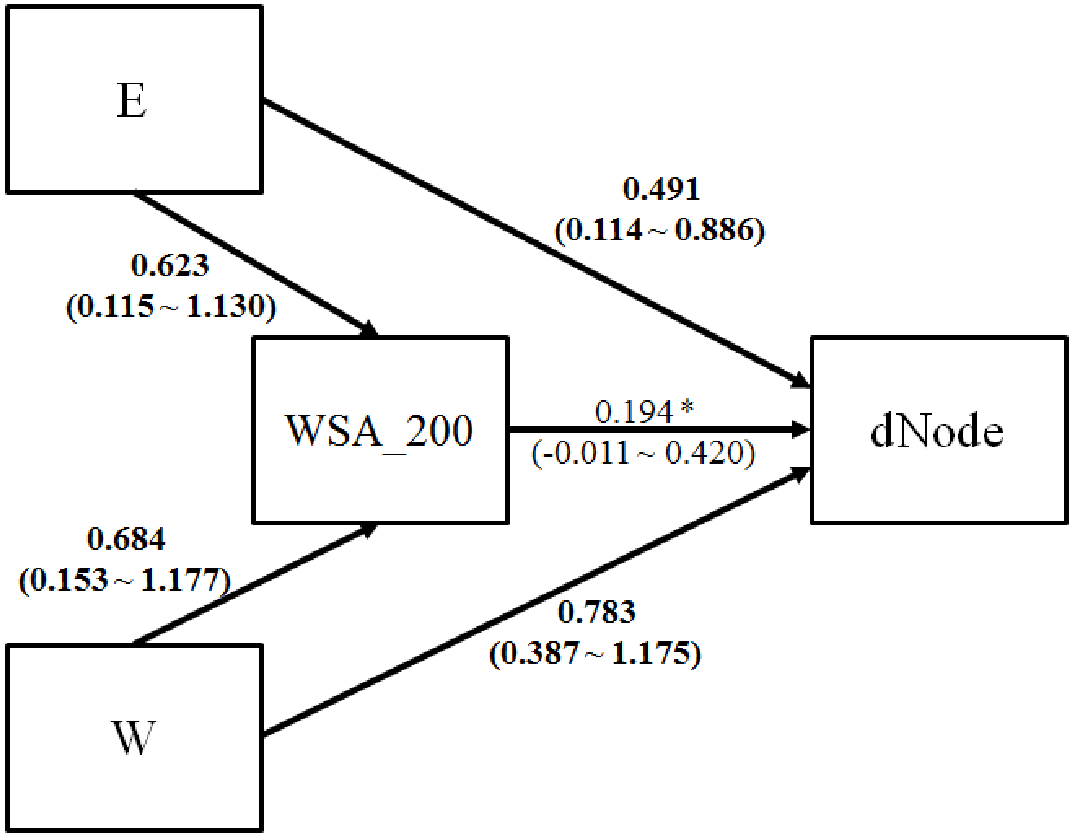


Fig. 10 DAG with standardized coefficients for how soil physical property mediating environmental effects on a plant trait (growth of node from week 2 to 6). Averaged and 95% CI of effect size for each path are shown. Posterior predicted p-value = 0.51 (not far from 0.5). Letters in bold indicates significantly different from 0 (95%). Letter with asterisk indicates marginally different from 0 (90%). dNode: difference in node number between week 2 to 6; E: earthworm treatment; W: under warming; WSA_200: water stable aggregate with size between 2.00 ~ 4.76 mm.

Appendix 1 Equations

$$\text{Paine's index} = \frac{T - C}{C} \qquad \text{(Eq. 1)}$$

T: trait value under treatment

C: trait value under control

$$C = -2\sum_{i=1}^{k} \ln(pi). \qquad \text{(Eq. 2)}$$

pi: probability of independence claim

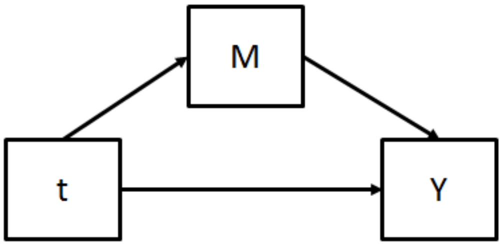
k: number of independence claim

$$\text{MWD} = \sum(X_i * WSA_i) \qquad \text{(Eq. 3)}$$

Xi: Size of sieve i

WSAi: percentage of water stable aggregate from sieve *i*

Representation for mediation analysis via potential outcome approach



Assume:

1. Y is a function of treatment (t) and mediator (M)
2. M is a function of treatment (t)

(Eq. 4)

For t = 0 (control), 1 (treated):

$$\delta_{\mathrm{i}}(\mathrm{t}) = \mathbb{E}(\mathrm{Yi}(\mathrm{t}, \mathrm{Mi}(1)) - \mathrm{Yi}(\mathrm{t}, \mathrm{Mi}(0)))$$
$$\zeta_{\mathrm{i}}(\mathrm{t}) = \mathbb{E}(\mathrm{Yi}(1, \mathrm{Mi}(\mathrm{t})) - \mathrm{Yi}(0, \mathrm{Mi}(\mathrm{t})))$$
$$\tau_{\mathrm{i}}(\mathrm{t}) = \mathbb{E}(\mathrm{Yi}(1, \mathrm{Mi}(1)) - \mathrm{Yi}(0, \mathrm{Mi}(0))) = \frac{1}{2}\sum_{t=0}^{1}\{\delta(t) + \zeta(t)\}$$

δ: averaged causal mediating (indirect) effect

ζ: averaged direct effect

τ: total effect

All potential outcome were acquired by expectation ($\mathbb{E}$) of several Monte Carlo draws.

Appendix 3 Supporting tables

Table S1. Statistical supports of candidates for mediator. Notation is the same as table S1.

Models	95% CI
N ~ E	0.096 ~ 0.393
A7 ~ N	0.351 ~ 1.267
B ~ E	0.210 ~ 0.320
A7 ~ B	0.009 ~ 0.069

Table S2. 95% confidence interval for all DAG tested in this study. Values in bold indicate statistically significant. Value with exclamation mark indicates marginally significant. E: earthworm treatment, W: warming treatment, N: leaf nitrogen content, B: number of branch, A3: aphid population in week 3, A7: aphid population in week 7, PC1: PC1 from 5 morphological trait in week 2, WSA_2: water stable aggregates 2.00 ~ 4.76 mm, dNode: growth of node from week 2 to 6.

DAG	ADE	ACME	Total	ACME / Total
E → N → A7	-0.095 ~ 0.457	**0.001 ~ 0.389**	**0.062 ~ 0.689**	-0.042 ~ 1.790 !
E → B → A7	-0.088 ~ 0.575	-0.033 ~ 0.273	**0.034 ~ 0.629**	-0.200 ~ 1.600
W → PC1 → A3	**-0.669 ~ -0.023**	-0.597 ~ 0.348	**-0.870 ~ -0.069**	-2.309 ~ 1.047
W → A3 → PC1	**-3.852 ~ -1.854**	-0.564 ~ 0.333	**-3.794 ~ -2.078**	-0.110 ~ 0.204
E → WSA_2. → dNode	**1.403 ~ 16.857**	**0.107 ~ 6.310**	**4.230 ~ 19.109**	**0.008 ~ 0.709**
W → WSA_2. → dNode	**7.031 ~ 21.276**	**0.253 ~ 6.794**	**9.527 ~ 24.517**	**0.013 ~ 0.389**

Appendix 3 WinBUGs code for hierarchical model with AR(1)

```
###hierarchical AR(1) random slope, intercept model for continuous data
## for plant traits
model;
{
for (i in 1:96){
Y1[i]~dnorm(lambda1[i],tau1)    #trait at week 2
Y2[i]~dnorm(lambda2[i],tau2)    # trait at week 4
Y3[i]~dnorm(lambda3[i],tau3)    # trait at week 6
mu1[i]<-alpha[1]+e.W1[W[i]]+e.R1[R[i]]
mu2[i]<-alpha[2]+e.W2[W[i]]+e.R2[R[i]]+e.E1[E[i]]+e.A1[A[i]]
mu3[i]<-alpha[3]+e.W3[W[i]]+e.R3[R[i]]+e.E2[E[i]]+e.A2[A[i]]
###trait at each week had different linear predictor (thus, a random intercept and slope model)
```

###AR(1) structure, the trait at next time point was correlated with last time point

```
lambda1[i]<-mu1[i]
lambda2[i]<-mu2[i]+gamma*(Y1[i]-mu1[i])
lambda3[i]<-mu3[i]+gamma*(Y2[i]-mu2[i])
}
```

##posterior predictive check

```
for (i in 1:96){
Y1.pred[i]~dnorm(lambda1[i],tau1)
Y1.res[i]<-abs(Y1[i]-lambda1[i])
Y1.res.rep[i]<-abs(Y1.pred[i]-lambda1[i])
Y1p.pred[i]<-step(Y1[i]-Y1.pred[i])
}
m.ppY1<-sum(Y1p.pred[])/96
fit.Y1<-sum(Y1.res[])
fit.Y1.rep<-sum(Y1.res.rep[])

for (i in 1:96){
Y2.pred[i]~dnorm(lambda2[i],tau2)
Y2.res[i]<-abs(Y2[i]-lambda2[i])
```

```
Y2.res.rep[i]<-abs(Y2.pred[i]-lambda2[i])
Y2p.pred[i]<-step(Y2[i]-Y2.pred[i])
}
m.ppY2<-sum(Y2p.pred[])/96
fit.Y2<-sum(Y2.res[])
fit.Y2.rep<-sum(Y2.res.rep[])

for (i in 1:96){
Y3.pred[i]~dnorm(lambda3[i],tau3)
Y3.res[i]<-abs(Y3[i]-lambda3[i])
Y3.res.rep[i]<-abs(Y3.pred[i]-lambda3[i])
Y3p.pred[i]<-step(Y3[i]-Y3.pred[i])
}
m.ppY3<-sum(Y3p.pred[])/96
fit.Y3<-sum(Y3.res[])
fit.Y3.rep<-sum(Y3.res.rep[])

###estimation for Paine's index
## average the random effect (experiment round) in this calculation
PW[1]<-(e.W1[2])/(alpha[1]+0.5*e.R1[2])
PW[2]<-(e.W2[2])/(alpha[2]+0.5*e.R2[2])
PW[3]<-(e.W3[2])/(alpha[3]+0.5*e.R3[2])

PE[1]<-(e.E1[2])/(alpha[2]+0.5*e.R2[2])
PE[2]<-(e.E2[2])/(alpha[3]+0.5*e.R3[2])
PA[1]<-(e.A1[2])/(alpha[2]+0.5*e.R2[2])
PA[2]<-(e.A2[2])/(alpha[3]+0.5*e.R3[2])

##priors (all settings were conjugate uninformative priors)
tau1~dgamma(0.005,0.005)
tau2~dgamma(0.005,0.005)
tau3~dgamma(0.005,0.005)
gamma~dnorm(0,1.0E-6)
for (i in 1:3){
alpha[i]~dnorm(alpha.mu,alpha.tau)}
```

```
e.W1[1]<-0;e.W2[1]<-0;e.W3[1]<-0;e.R1[1]<-0;e.R2[1]<-0;
e.R3[1]<-0;e.E1[1]<-0;e.E2[1]<-0;e.A1[1]<-0;e.A2[1]<-0;
e.W1[2]~dnorm(W.mu,W.tau)
e.W2[2]~dnorm(W.mu,W.tau)
e.W3[2]~dnorm(W.mu,W.tau)
e.R1[2]~dnorm(R.mu,R.tau)
e.R2[2]~dnorm(R.mu,R.tau)
e.R3[2]~dnorm(R.mu,R.tau)
e.E1[2]~dnorm(E.mu,E.tau)
e.E2[2]~dnorm(E.mu,E.tau)
e.A1[2]~dnorm(A.mu,A.tau)
e.A2[2]~dnorm(A.mu,A.tau)

##hyper-priors (second level)
alpha.mu~dnorm(0,1.0E-6)
W.mu~dnorm(0,1.0E-6)
R.mu~dnorm(0,1.0E-6)
E.mu~dnorm(0,1.0E-6)
A.mu~dnorm(0,1.0E-6)
alpha.tau~dgamma(0.005,0.005)
W.tau~dgamma(0.005,0.005)
R.tau~dgamma(0.005,0.005)
E.tau~dgamma(0.005,0.005)
A.tau~dgamma(0.005,0.005)
}
```

Appendix IV Presentation of raw data

All raw data are presented as data accumulation graph. Each variable was normalized by ((Xi – min) / (max-min)). Each value after this normalization ranged from 0 to 1. The values of each trait which belonged to the same observation were added up in stacked histogram. Each bar represented one observation.

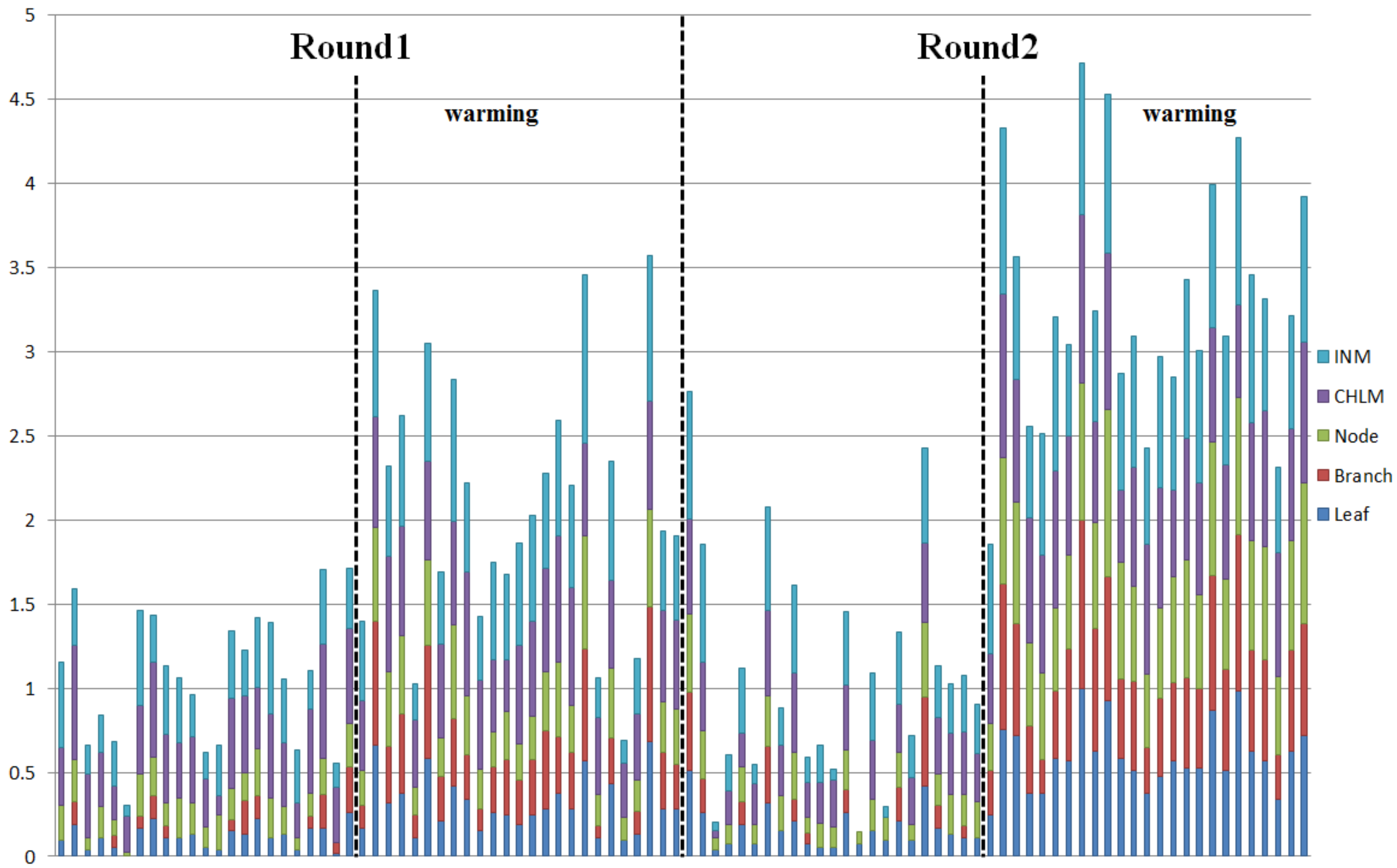


Figure S1 Data Accumulation graph for plant traits in week 3. The 96 observations were shown and separated by experimental rounds and warming treatment.

Data Accumulation graph 1 – plant traits in week 3

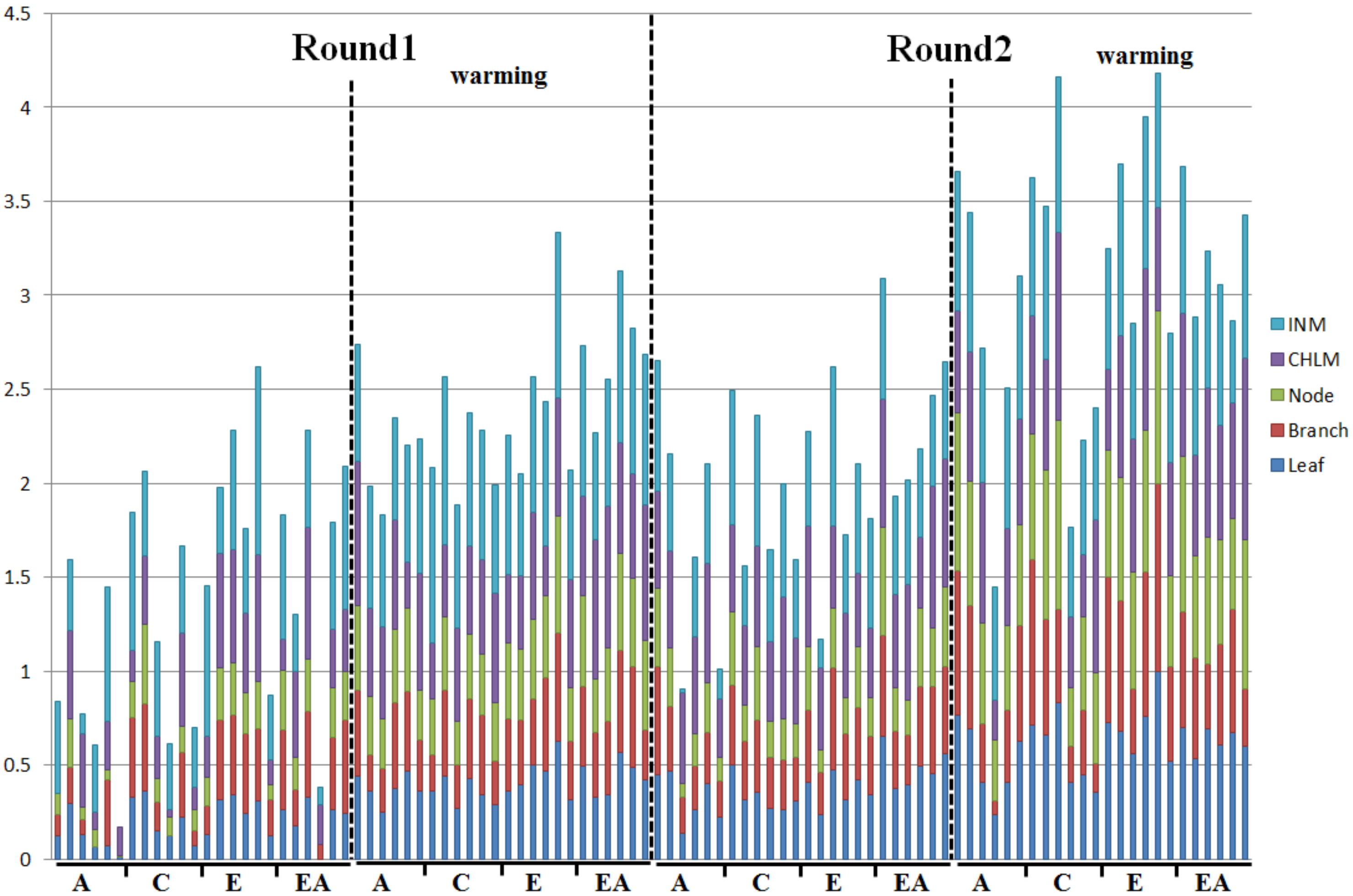


Figure S2 Data Accumulation graph for plant traits in week 5. The species combination of treatments was shown in the bottom. A: aphid/ C: control/ E: earthworm/ EA: earthworm & aphid

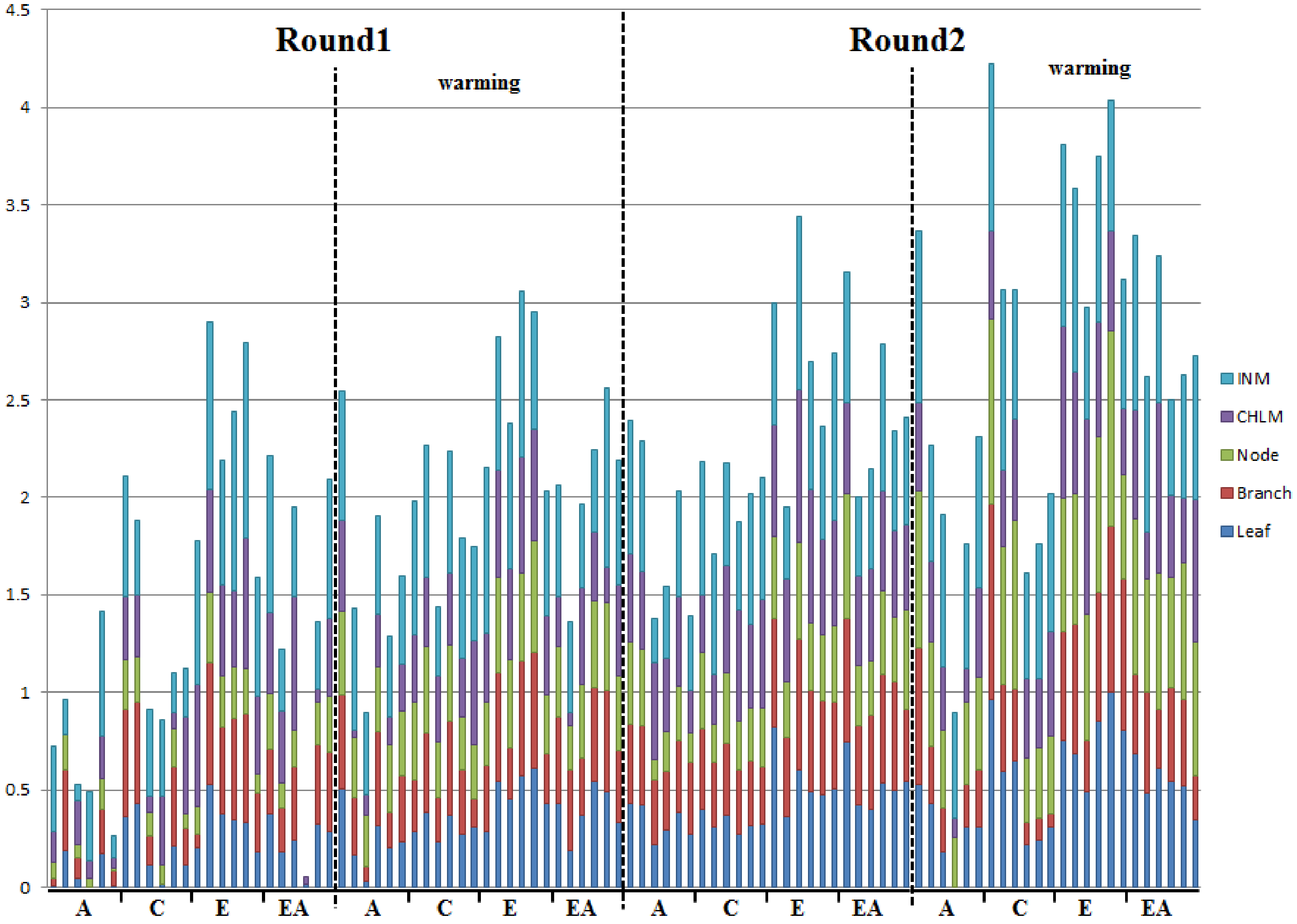


Figure S3 Data Accumulation graph for plant traits in week 7.

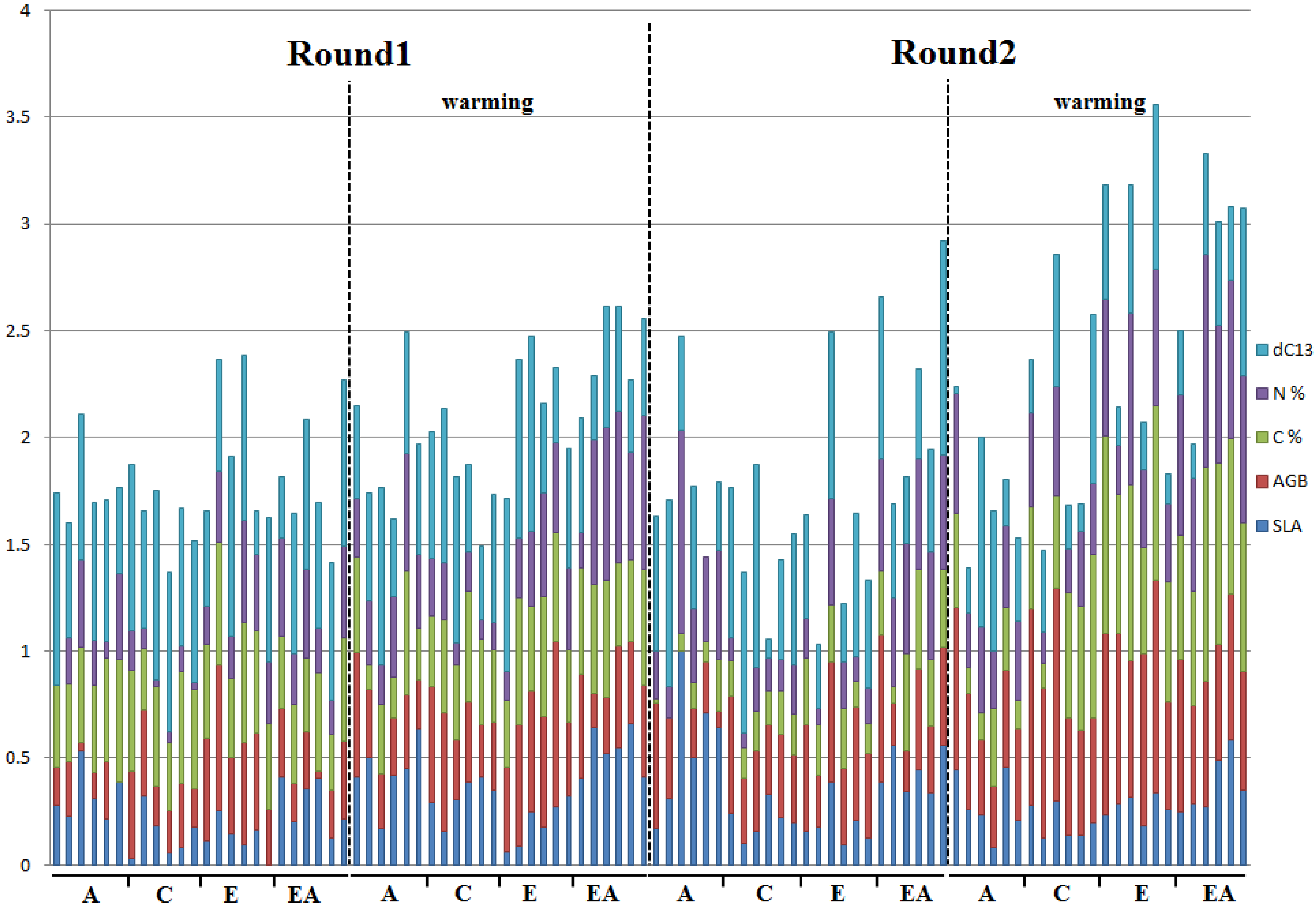


Figure S4 Data Accumulation graph for plant traits obtained by destructive sampling .

Table S3 Minimum and maximum values of repeated measured plant traits in week 3, 5, and 7 and plant traits by destructive sampling.

	Leaf3	Branch3	Node3	CHLM3	INM3
min	14	5	9	25.7	1.9
max	67	20	52	46.2	6.95

	Leaf5	Branch5	Node5	CHLM5	INM5
min	26	8	20	26	3.85
max	133	34	107	48.2	7.9

	Leaf7	Branch7	Node7	CHLM7	INM7
min	34	9	29	17.1	3.725
max	148	36	152	44.4	7.65

	SLA	AGB	C %	N %	ƹ C13
min	158.867	0.519	35.95	1.00	-31.2
max	352.009	5.846	40.77	2.32	-29.2

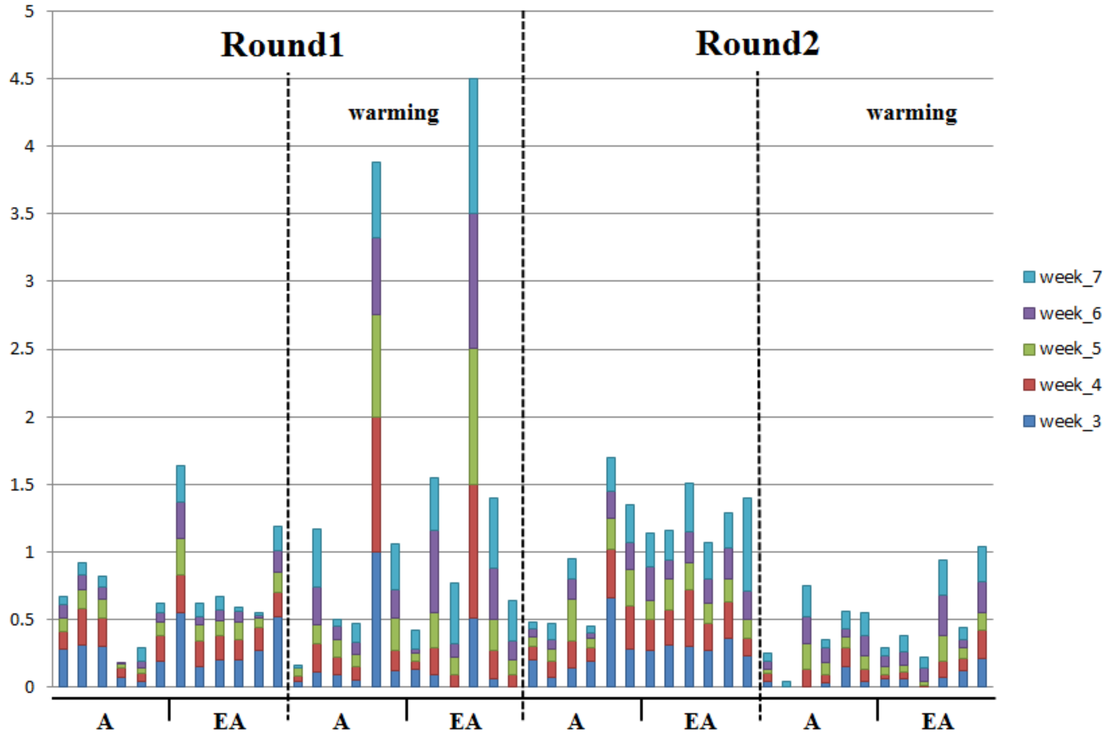


Figure S5 Data Accumulation graph for aphid population from week 3 to 7.

Table S4 The minimum and maximum values of the aphid populations from week 3 to 7

	Week3	Week4	Week5	Week6	Week7
min	10	10	67	415	955
max	105	485	2475	6895	8880

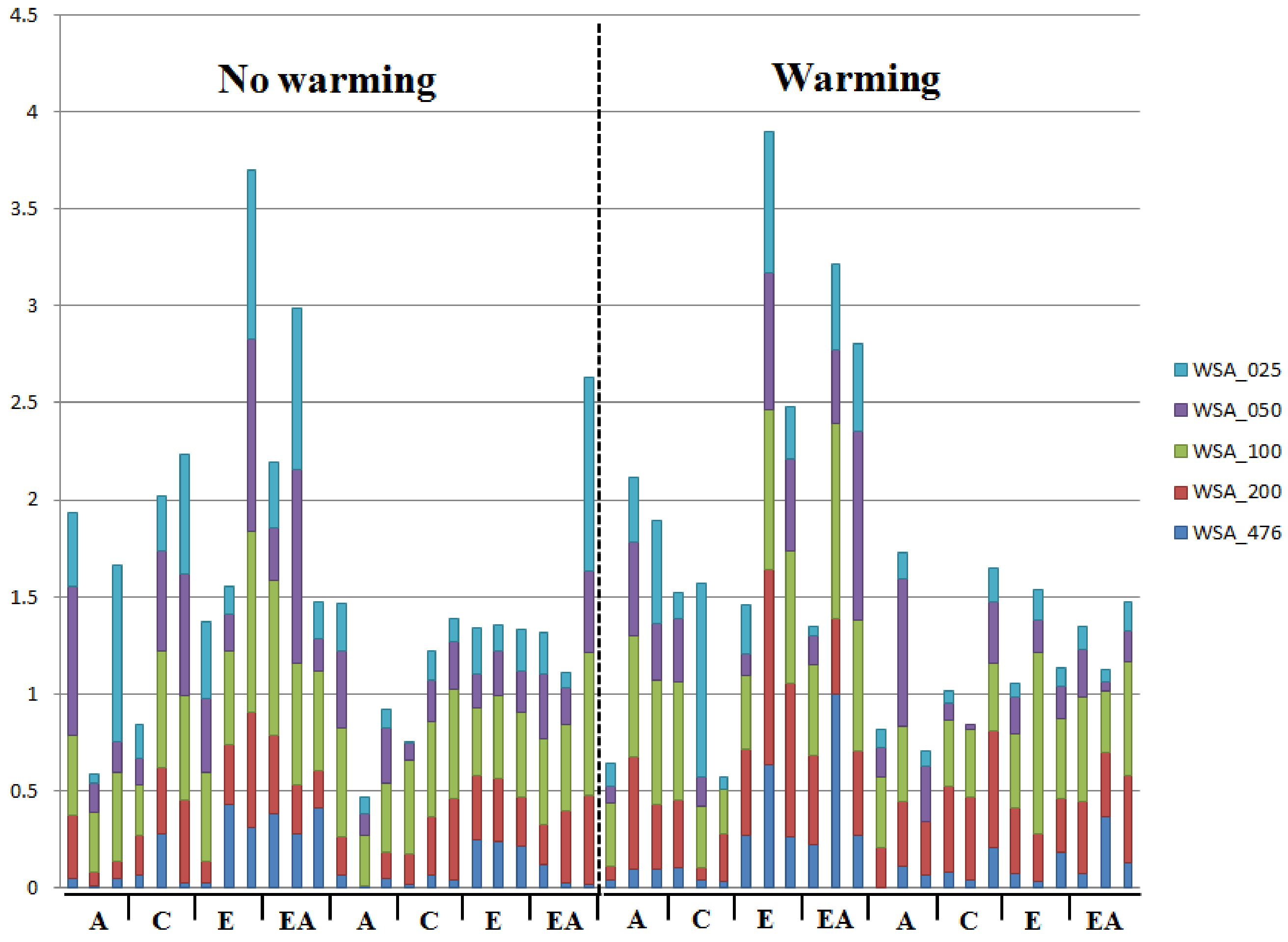


Figure S5 Data Accumulation graph for water stable soil aggregate distribution.

Table S5 The minimum and maximum values in water stable soil aggregate distribution

	WSA_476	WSA_200	WSA_100	WSA_050	WSA_025
min	0.00	0.37	0.00	1.13	2.05
max	12.87	3.84	2.35	5.38	11.71

Table S6 Earthworm survival and weight (IFW: initial fresh weight, SUR: number of earthworm survived, FFW: final fresh weight)

ID	IFW	SUR	FFW	ID	IFW	SUR	FFW
ANE1	4.69	2	3.98	BNE1	2.365	1	1.43
ANE2	3.23	1	1.58	BNE2	1.745	2	1.935
ANE3	1.44	1	0.9	BNE3	2.045	2	2.255
ANE4	4.42	2	3.78	BNE4	2.765	1	1.43
ANE5	3.56	2	2.485	BNE5	1.94	0	NA
ANE6	2.02	2	1.81	BNE6	2.045	0	NA
ANEa1	3.7	2	3.83	BNEa1	4.515	2	4.22
ANEa2	1.74	1	1.065	BNEa2	3.12	2	3.015
ANEa3	2.35	2	2.25	BNEa3	2.195	1	1.09
ANEa4	1.88	0	NA	BNEa4	1.88	2	1.84
ANEa5	3.88	1	1.8	BNEa5	2.745	1	2.105
ANEa6	2.89	2	3.035	BNEa6	2.595	1	1.865
BWE1	2.18	0	NA	CWE1	3.985	2	3.17
BWE2	2.806	1	0.985	CWE2	2.75	2	2.135
BWE3	2.13	2	1.815	CWE3	2.955	2	2.27
BWE4	2.25	1	1.385	CWE4	3.11	2	2.61
BWE5	2.57	2	2.6	CWE5	2.855	1	0.625
BWE6	3.16	2	2.55	CWE6	2.985	2	2.465
BWEa1	3.39	2	3.03	CWEa1	2.78	2	2.685
BWEa2	3.66	2	2.985	CWEa2	2.765	2	2.25
BWEa3	3.32	2	2.695	CWEa3	1.64	0	NA
BWEa4	3.075	2	2.89	CWEa4	1.935	2	1.97
BWEa5	3.1	2	2.475	CWEa5	2.43	2	2.495
BWEa6	2.215	1	0.555	CWEa6	2.345	0	NA