

SCIENTIFIC REPORTS



OPEN

Community diversity, structure and carbon footprint of nematode food web following reforestation on degraded Karst soil

Received: 12 January 2016

Accepted: 27 May 2016

Published: 17 June 2016

Ning Hu^{1,2,*}, Hui Li^{3,*}, Zheng Tang^{4,*}, Zhongfang Li^{4,*}, Jing Tian⁵, Yilai Lou², Jianwei Li⁶, Guichun Li² & Xiaomin Hu¹

We examined community diversity, structure and carbon footprint of nematode food web along a chronosequence of *T. Sinensis* reforestation on degraded Karst. In general, after the reforestation: a series of diversity parameters and community indices (Shannon-Weiner index (H'), structure index (SI), etc.) were elevated; biomass ratio of fungivores to bacterivores (FFC/BFC), and fungi to bacteria (F/B) were increased, and nematode channel ratio (NCR) were decreased; carbon footprints of all nematode trophic groups, and biomass of bacteria and fungi were increased. Our results indicate that the Karst aboveground vegetation restoration was accompanied with belowground nematode food web development: increasing community complexity, function and fungal dominance in decomposition pathway, and the driving forces included the bottom-up effect (resource control), connectedness of functional groups, as well as soil environments.

Karst terrains formed from carbonate minerals account for about 15% of the world's land area¹. The unique ecosystem is very fragile and sensitive, with a low environmental capacity², and the soil is thin, coarse, highly erosive and degenerative³. Karst soil covering approximately 0.55 million km² in southwest China, has been subjected to intensive anthropogenic disturbances (e.g., cultivation, deforestation, grazing and burning)^{4,5}. These disturbances rapidly expand after 1970s because of the pressure from the increasing population and land overuse^{6,7}. This accelerates Karst ecosystem degradation especially of rocky-desertification⁸. The degradation adversely affects soil fertility and results in the genesis of abandoned bare land. Such degraded land needs proper ecological restoration through which soil can be ameliorated to support biological productivity⁹. Reforestation, as one method by which degraded sites can be restored back to maintain soil fertility⁹, has been increasingly adopted in Chinese Karst regions since 1990s, and *T. Sinensis* is one of the commonly used tree species.

Soil nematode communities are useful biological indicators of soil health, because they form a dominant group of soil organisms and live in various types of soils¹⁰. These communities also represent key links in soil food webs, such as plant-parasite, bacterivores, fungivores, omnivores and predators, and their trophic structures are closely correlated with soil ecosystem processes¹⁰. Nematode community size and complexity can reflect the vegetation change and are regarded as sensitive bio-indicators of ecosystem restoration¹¹. Microbiovorous nematodes, as consumers of microflora, indirectly regulate decomposition and release of nutrients in ecosystems¹⁰. The relative importance of bacterivores vs. fungivores is closely related to that of bacteria vs. fungi, and the relevant indices such as fungivores to bacterivores ratio (FF/BF) and nematode channel ratio (NCR) are extensively used to indicate the decomposition pathway of soil food web¹². Recently, there has been an increasing interest in the

¹College of Resources and Civil Engineering, Northeastern University, 110819 Shenyang, China. ²Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, 100081 Beijing, China. ³College of Resources and Environmental Sciences, Henan Agricultural University, 450002 Zhengzhou, China. ⁴College of Chemistry and Bioengineering, Hezhou University, 542899 Hezhou, China. ⁵Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, 100101 Beijing, China. ⁶National Engineering Laboratory for Improving Quality of Arable Land, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, 100081 Beijing, China. *These authors contributed equally to this work. Correspondence and requests for materials should be addressed to Y.L. (email: louyilai@caas.cn) or X.H. (email: hxmin_jj@163.com)

	0-yr	4-yr	8-yr	16-yr
Porosity (%)	31.41 ± 2.65 c	33.62 ± 2.61 bc	38.41 ± 3.42 ab	40.24 ± 3.10 a
Moisture (%)	11.42 ± 1.74 c	11.91 ± 2.23 bc	15.12 ± 1.86 ab	16.22 ± 1.83 a
TOC (g kg ⁻¹)	11.94 ± 1.17 c	13.12 ± 1.20 bc	15.61 ± 3.32 ab	17.54 ± 2.21 a
TN (g kg ⁻¹)	1.21 ± 0.10 b	1.22 ± 0.07 b	1.29 ± 0.13 ab	1.37 ± 0.05 a
C:N	9.85 ± 0.53 b	10.74 ± 1.21 ab	12.12 ± 1.83 ab	12.83 ± 1.69 a
pH	6.72 ± 0.28	6.79 ± 0.17	6.91 ± 0.21	6.82 ± 0.09

Table 1. Soil basic properties along a chronosequence of *T. Sinensis* reforestation (mean ± standard deviation, $n = 4$). Different letters indicate significant difference between treatments at $P < 0.05$. TOC, total organic carbon; TN, total nitrogen; C:N, ratio of TOC to TN.

metabolic activity of nematode communities, which provides information on the magnitude or nature of ecosystem functions^{13–15}. Ferris suggested using the nematode metabolic footprint to extend ecosystem assessments¹³. This footprint conveys additional information on the biomass, metabolic activity, and magnitudes of carbon (C) and energy flow in soil food webs, and provides an effective method for monitoring the available resources and estimating the contribution of nematodes to ecosystem services and functions¹¹.

Recently, nematode community characteristics were reported in the Karst regions^{16–18}. However, to date, nematodes have been poorly known following *T. Sinensis* reforestation on the degraded Karst soil. Reforestation can increase vegetation diversity and belowground resource input and can improve soil environments such as moisture and porosity^{2,19}. Consequently, the *T. Sinensis* reforestation on Karst may increase nematode community size, complexity and carbon footprint. Additionally, the *T. Sinensis* reforestation can alter the type of resource input, and therefore may change the resource quality and in turn may influence nematode food web structure and decomposition pathway. The main objective of this study was to examine diversity, structure and carbon footprint of nematode food web in response to *T. Sinensis* reforestation on the degraded Karst soils.

Results

Soil basic properties. There were significant differences in all the studied soil environmental variables (except for pH) among the different ages of forests ($P < 0.05$) (Table 1). These variables showed an increasing trend with the reforestation age. To the 16-yr age, soil porosity, moisture, TOC content, TN content, and C:N increased by 28.1%, 42.0%, 46.9%, 13.2% and 30.3%, respectively, compared with the 0-yr control.

Nematode community composition. At the 0-yr control site, some genera (such as *Alaimus*, *Tylencholaimus*, *Rotylenchulus*, *Axonchium*, *Microdorylaimus* and *Mesodorylaimus*) were inexistent and only 23 genera were recorded (Table 2). After reforestation, these inexistent genera gradually appeared and the recorded genera number increased to 34 of the 16-yr reforestation. The dominant genera were *Acrobeloides*, *Rhabdolaimus* and *Aphelenchoides* for all the four study sites, and their relative abundances all decreased with the reforestation age. A redundancy analysis (RDA) showed that the composition of nematode community was clearly discriminated among the different ages of forests, and showed that soil basic properties of moisture, porosity and TOC were closely related to the distribution of nematode genera (Fig. 1). The eigenvalues were 0.131 ($F = 3.412$, $P = 0.003$) and 0.242 ($F = 2.157$, $P = 0.002$) for the first canonical axis and all canonical axes, respectively, and the first two axes explained 53.3% of the variation.

Nematode diversity and food web structure. All measured community indices significantly differed among the four study sites in both seasons ($P < 0.05$) (Table 3). The values of H' , SR, and J' all tended to increase with the reforestation age, and to the 16-yr reforestation increased by 44.9%, 41.0% and 19.4%, respectively, in June, and by 32.3%, 38.2% and 16.0%, respectively, in September. The values of MI and SI were both greater in the older forests than the younger forests in both seasons. After the 16-yr reforestation the NCR value decreased by 28.2%, compared with the 0-yr control in both seasons. The FF/BF ratio and F/B ratio both showed an increasing tendency with the reforestation age in both seasons.

Community size and biomass carbon. In general, the abundance and biomass carbon of all nematode trophic groups significantly increased with the reforestation age ($P < 0.05$) (Fig. 2). On average, the abundance and biomass carbon of total nematode increased at a rate of 19 individuals $100\text{ g}^{-1}\text{ yr}^{-1}$ and $267\text{ }\mu\text{g kg}^{-1}\text{ yr}^{-1}$, respectively, in June, and of 17 individuals $100\text{ g}^{-1}\text{ yr}^{-1}$ and $236\text{ }\mu\text{g kg}^{-1}\text{ yr}^{-1}$, respectively, in September. The consistent increasing trend was also found for the biomass of bacteria, fungi, and total microbes in both seasons (Fig. 3). The final structural equation model (SEM) on the bottom-up effect of the nematode food web adequately fit the data and the standardized path coefficients ($\chi^2 = 8.472$, $df = 14$, $P = 0.649$, CFI = 0.988, IFI = 0.971, RMSEA = 0.002) (Fig. 4). Root biomass (R) was significantly correlated to the biomass of bacteria (B), fungi (F), and plant-parasites (PP); the B and F were significantly correlated to the biomass of bacterivores (BF) and fungivores (FF), respectively; and the BF, FF, F and PP were all significantly correlated to the biomass of omnivores-predators (OP). The model explained 71%, 64%, 69% and 82% of the variance in BF, FF, PP, and OP, respectively.

Discussion

In our study, a RDA analysis indicated that the nematode communities of different sites clustered corresponding to forest age. The nematode community composition change might be directly related to the vegetation restoration. The vegetation coverage and diversity increased after the *T. Sinensis* reforestation in our study. Additionally,

Genus	Abbr.	0-yr	4-yr	8-yr	16-yr
Bacterivores					
<i>Diplogasteriana</i>	<i>Dip</i>	0.7	1.2	1.9	2.9
<i>Protorhabditis</i>	<i>Pro</i>	0.9	2.4	2.4	3.3
<i>Rhabditonema</i>	<i>Rha</i>	0.4	1.9	2.2	3.6
<i>Acrobeles</i>	<i>Acr</i>	6.4	2.2	1.1	0.9
<i>Heterocephalobus</i>	<i>Het</i>	1.5	1.2	0.9	1.8
<i>Pseudacrobeles</i>	<i>Pse</i>	1.5	1.3	1.2	1.6
<i>Teratocephalus</i>	<i>Ter</i>	1.5	1.4	0.7	1.6
<i>Eucephalobus</i>	<i>Eur</i>	0.8	0.7	0.7	1.1
<i>Acrobeloides</i>	<i>Acr</i>	21.2	18.7	12.6	10.1
<i>Tylocephalus</i>	<i>Tyl</i>	1.2	1.0	0.9	1.1
<i>Prismatolaimus</i>	<i>Pri</i>	0.9	0.7	1.5	0.9
<i>Rhabdolaimus</i>	<i>Rhab</i>	29.1	23.0	14.3	11.2
<i>Alaimus</i>	<i>Ala</i>	0.0	2.0	2.2	2.3
Fungivores					
<i>Aphelenchus</i>	<i>Aph</i>	1.9	5.9	7.9	9.6
<i>Aphelenchoides</i>	<i>Aphe</i>	16.7	15.9	15.2	10.4
<i>Filenchus</i>	<i>Fil</i>	1.4	5.5	7.8	8.4
<i>Tylencholaimus</i>	<i>Tyle</i>	0.0	0.6	4.2	6.7
Plant-parasites					
<i>Atylenchus</i>	<i>Aty</i>	2.3	1.8	1.4	0.7
<i>Lelenchus</i>	<i>Lel</i>	2.3	1.8	1.1	0.6
<i>Tylenchus</i>	<i>Tylen</i>	1.9	1.7	1.1	0.5
<i>Paratylenchus</i>	<i>Par</i>	2.2	1.7	1.2	0.6
<i>Criconemella</i>	<i>Cri</i>	0.0	0.5	0.4	0.5
<i>Pratylenchus</i>	<i>Pra</i>	0.8	0.8	0.5	1.1
<i>Rotylenchulus</i>	<i>Rot</i>	0.0	0.0	0.5	1.0
<i>Rotylenchus</i>	<i>Roty</i>	0.0	0.0	0.6	0.7
<i>Heterodera</i>	<i>Hete</i>	2.6	2.8	2.2	2.1
<i>Axonchium</i>	<i>Axo</i>	0.0	0.9	1.3	1.8
<i>Dorylaimellus</i>	<i>Dor</i>	0.0	1.1	1.3	1.9
Omnivores-Predators					
<i>Thonus</i>	<i>Tho</i>	0.8	0.7	1.5	1.6
<i>Eudorylaimus</i>	<i>Eud</i>	0.9	0.7	1.6	2.1
<i>Microdorylaimus</i>	<i>Mic</i>	0.0	0.0	1.8	1.8
<i>Discolaimus</i>	<i>Dis</i>	0.0	0.0	0.0	1.6
<i>Mesodorylaimus</i>	<i>Mes</i>	0.0	0.0	1.6	1.9
<i>Prodorylaimium</i>	<i>Prod</i>	0.0	0.0	4.2	2.0

Table 2. Seasonally mean relative abundance (%) of nematode genera along a chronosequence of *T. Sinensis* reforestation.

soil basic properties can control nematode communities^{20,21}. Our RDA analysis showed that soil TOC content, porosity and moisture were closely related to nematode genera distribution. In the present study, a serious nematode ecological indices (genus number, species richness index (SR), evenness index (J') and Shannon-Weiner diversity index (H'), maturity index (MI), and structure index (SI)) generally increased with the reforestation age. These results indicated that the *T. Sinensis* reforestation created the more diverse and structured nematode community. The *T. Sinensis* reforestation increased belowground resource input (as indicated by the elevated root biomass) and improved soil environments (as suggested by the increased porosity and moisture, etc.), and therefore the relatively complicated and mature soil food web was developed²². Additionally, generally there is a good association of aboveground and belowground biodiversity^{23,24}. The increased nematode diversity might be directly resulted from the increased vegetation species diversity in our study. Consistently with our results, Guan *et al.*²⁵ showed a gradually increasing trend in nematode community complexity with increasing age of *Caragana microphylla*. However, another study reported a different pattern that the most complicated nematode community was not found in the old but the mid-age forest. Kardol *et al.*²⁶ showed that restoration of aboveground communities is of limited indicative value for belowground developments: successful restoration of vegetation diversity does not necessarily imply successful restoration of belowground diversity. The different patterns of nematode diversity in a vegetation chronosequence may depend on different vegetation types, time scales, and study sites.

The extent to which decomposition is fungal-mediated can be reflected by the structure of the microbial feeding nematode community^{27,28}. In our study, as expected, the nematode channel ratio (NCR) tended to decline

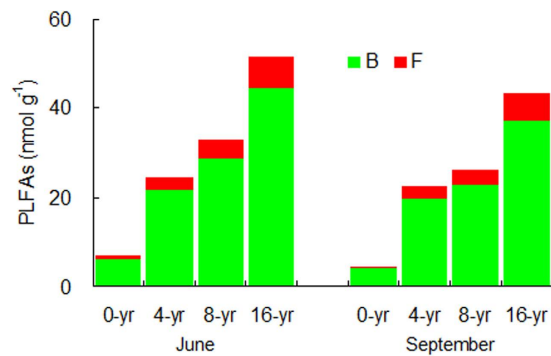


Figure 3. Mean phospholipid fatty acids (PLFAs) contents of bacteria (B) and fungi (F) along a chronosequence of *T. Sinensis* reforestation ($n = 4$).

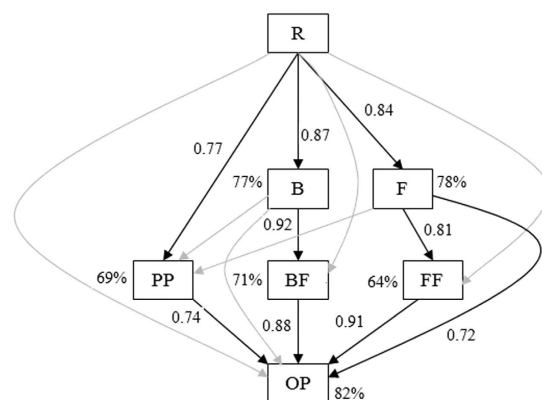


Figure 4. Structural equation model of bottom-up effect of the nematode food web ($\chi^2 = 8.472$, $df = 14$, $P = 0.649$, $CFI = 0.988$, $IFI = 0.971$, $RMSEA = 0.002$). Numbers next to the arrows are the standardized path coefficients. Black arrows indicate significant relationships ($P < 0.05$). Grey arrows indicate non-significant relationships or paths removed to optimize model fits. Percentages close to endogenous variables indicate the variance explained by the model. R: root biomass; B and F: biomass of bacteria and fungi, respectively; BF, FF, PP and OP: biomass carbon of bacterivores, fungivores, plant-parasites and omnivores-predators, respectively.

the food web through fungivorous channels than bacterivorous channels in older forests. The biomass ratio of fungi to bacteria (F/B) also showed the increasing trend. This can be explained by the change in the belowground resource quality. In our study, the lignin content and C/N ratio of the root tended to increase, while N content tended to decrease after the reforestation. This indicated that the more recalcitrant resource was formed and this therefore favored the fungal dominance in decomposition channel. Increasing soil aggregation is usually associated with greater fungal activity^{29,30}. It is known that fungi are aerobic organisms³⁰, and thus great aeration may favor fungal-mediated decomposition³¹. In our investigation, the increased soil porosity means the increased soil aggregation and aeration, and this so might contribute to the growing importance of fungal mediation in decomposition to some extent after the reforestation. Similar decomposition pathway results were also reported in other studies^{12,26,32–34}. These findings were in agreement with succession from bacterivory to fungivory often found in nematode faunas^{35,36} and with a general view of predominance of a fungus-based decomposition channel in advanced successional stages³⁷.

Consistently with abundance, biomass carbon of total nematode and trophic groups all increased with reforestation age in our study. The nematode trophic biomass indicated the C and energy flow into the soil food web through their respective trophic channels¹³. The biomass of bacteria, and fungi also increased. These results demonstrated that after the *T. Sinensis* reforestation the community size and function of the nematode food web. The bottom-up effect (resource control) generally occurs in soil food web^{11,38}. The predator-prey channel was one of the primary channels of the soil micro-food web³⁹, and the flow of C and energy through the soil food web is mainly driven by the feeding interrelationship among soil biota communities^{38,40}. In our structural equation model (SEM), root biomass (R) directly affected the biomass of bacteria (B), fungi (F), and plant-parasites (PP); the B and F significantly affected the biomass of bacterivores (BF) and fungivores (FF), respectively; and the F, BF, FF and PP together influenced the biomass of omnivores-predators (OP). These data confirmed that bacterivores and fungivores both played important roles in the C and energy flow between microbes and nematodes, and bacteria and fungi acted as primary prey for bacterivores and fungivores, respectively; that fungi, bacterivores,

	0-yr	4-yr	8-yr	16-yr
Vegetation				
Spices number (100 m ⁻²)	3.62 ± 0.25 d	12.41 ± 1.02 c	18.55 ± 1.46 b	22.25 ± 1.79 a
Cover degree (%)	5.12 ± 0.35 c	34.66 ± 2.12 b	37.51 ± 3.12 b	46.31 ± 3.75 a
Belowground resource				
Root biomass (g m ⁻²)	41.47 ± 5.17 c	291.1 ± 28.2 b	328.2 ± 29.4 b	416.1 ± 27.2 a
C content (%)	40.21 ± 3.12	41.22 ± 2.97	44.31 ± 4.12	41.15 ± 3.01
N content (%)	1.42 ± 0.12 a	1.04 ± 0.09 b	0.86 ± 0.04 c	0.82 ± 0.05 c
C/N	28.32 ± 2.51 c	39.63 ± 2.77 b	51.52 ± 4.81 a	50.18 ± 4.64 a
Cellulose content (%)	26.72 ± 1.58	26.19 ± 2.23	24.92 ± 2.19	27.82 ± 2.46
Lignin content (%)	12.41 ± 1.21 c	21.56 ± 1.74 b	22.22 ± 1.88 b	29.47 ± 2.14 a

Table 4. Information on aboveground vegetation and belowground resource input (0–10 cm depth) along a chronosequence of *T. Sinensis* reforestation (mean ± standard deviation, $n = 4$). Different letters indicate significant difference between treatments at $P < 0.05$.

fungivores and plant-parasites were primary prey for omnivores-predators; and that the resource input controlled the nematode food web. The variance in the food web unexplained by the model possibly attributed to soil environmental variables such as moisture and porosity. Our results indicate that the bottom-up effect from the vegetation, the interactions of the functional groups, as well as soil environments together were the drivers of the reforested Karst soil food web.

Methods

Study site and experiment design. This study was conducted at Huanjiang county (107°54' E, 24°49' N), Guangxi province in China, with typical Karst ecosystems. This region belongs to a subtropical monsoon climate with mean annual precipitation of 1,389 mm and mean annual temperature of 19.9°C. The calcareous soil developed from a dolostone base⁴¹. The soil degradation is serious due to the intensive anthropogenic disturbance especially of cultivation and therefore results in a large area of abandoned bare land in this region. Since 1990s, large scales of *T. Sinensis* forestation have been adopted to restore the degraded ecosystem. Three *T. Sinensis* forestations (4-, 8-, and 16-yr) were selected as the experimental sites while a nearby abandoned bare land as the 0-yr control site. The four sites were selected to show very similar altitude, topography, vegetation background and disturbance experience (over 40 years of cultivation) before forestation and the same soil type. Each site has a size of over 10,000 m², and four plots (40 m × 40 m) as replications were selected within each site. The distance between plots within a site was about 10 m. The *T. Sinensis* was planted at a 7 m × 5 m density. We investigated characteristics of vegetation and belowground resources in June 2015. The tree diameter and height averaged 5 cm and 2.54 m, 11 cm and 2.59 m, 19 cm and 2.68 m for the 4-, 8-, and 16-year forestations, respectively. The vegetation species diversity and coverage degree showed an increasing trend with the reforestation age (Table 4). The resource input for the belowground soil food web was mainly from the turnover of the roots (including the fine roots of the shrub and tree and the herbaceous root). We used the excavation method (10 cm × 10 cm × 10 cm) to collect the roots, and then measured the root biomass and chemistry. The root cellulose and lignin contents were measured using the colorimetric method and Klason method, respectively⁴², and the root C and N contents were determined using an element Analyzer (Elementar, Germany). The root biomass significantly increased with the reforestation age, and the root chemistry notably differed among the sites: the lignin content and C/N tended to increase, while the N content tended to decrease with the reforestation age (Table 4). Ten soil cores (2.5 cm diameter) were collected to a depth of 10 cm depth along an S-shaped transect using an auger and were mixed to form one composite sample, after excluding litter and humus layer from each plot in both seasons of June and September 2015.

Nematode community analysis. Nematodes were extracted from 100 g field moist soil by a modified cotton-wool filter method¹¹. After counting the total number of nematodes in a sample, 100 individuals were randomly selected and identified to genus level using an inverted compound microscope according to Bongers⁴³ and Ahmad and Jairjuri⁴⁴. If the total nematodes did not reach 100 in a sample, all the nematodes in the sample were identified. Nematode abundance was expressed as individuals per 100 g of dry soil. Nematodes were assigned to the following trophic groups according to their feeding habits: bacterivores (BF), fungivores (FF), omnivores-predators (OP) and plant-parasites (PP)⁴⁵.

Following identification, the length (L) and maximum body diameter of all nematodes were measured using an ocular micrometer. Nematode biomass was calculated using the following formula:

$$W = (L^3/a^2)/(1.6 \times 10^6) \quad (1)$$

where W is the fresh weight (μg) per taxon, L is nematode length (μm), and a is the ratio of length to maximum body diameter⁴⁵. Total nematode biomass C was estimated by multiplying the abundance of each taxon by their calculated fresh weight⁴⁶, using a fresh weight/dry weight conversion factor of 0.20⁴⁷ and a C content of 52% of dry weight⁴⁸.

The Shannon-Weiner diversity index (H'), species richness index (SR) and evenness index (J') were used as the indication of soil nematode diversity and were calculated using the following formulae:

$$H' = -\sum p_i \ln(p_i) \quad (2)$$

where p_i is the proportion of individuals in the i th taxon⁴⁹;

$$SR = (S - 1)/\ln(N) \quad (3)$$

where S is the total number of genera and N is the total number of individuals in the community⁵⁰;

$$J' = H'/\ln(S) \quad (4)$$

where H' is Shannon-Weinier index and S is the total number of genera⁵¹. Community indices of nematode channel ratio (NCR), maturity index (MI) and structure index (SI) were calculated as follows:

$$\text{NCR} = B/(B + F) \quad (5)$$

where B and F are the numbers of bacterivores and fungivores in the total nematode community, respectively¹²;

$$\text{MI} = (\sum v_i f_i)/N \quad (6)$$

where v_i is the c-p value assigned to genus i , f_i is the frequency of genus i , and N is the total number of individuals in the community⁵²;

$$\text{SI} = 100 \times s/(b + s)^{27} \quad (7)$$

where b and s are the abundance of individuals in guilds in the basal component and structural component weighted by their k_b and k_s values, respectively. k_b is the weighting assigned to guilds Ba_2 and Fu_2 , and k_s is the weighting assigned to guilds Ba_3 – Ba_5 , Fu_3 – Fu_5 , and Op_3 – Op_5 . Ba_x , Fu_x , Op_x and Pp_x (where $x = 1$ – 5) represent the functional guilds of nematodes that are bacterivores (Ba), fungivores (Fu), omnivores-predators (Op) or plant parasites (Pp), respectively, where the guilds have the character indicated by x on the colonizer-persister (c-p) scale (1–5) according to their r and K characteristics.

Measurement of microbes and soil basic properties. Phospholipid fatty acids (PLFAs) analysis was performed using the method described by Helgason *et al.*⁵³. Briefly, fatty acids were extracted from 4 g freeze-dried soils using a single phase chloroform, methanol, phosphate buffer solution. The isolated fatty acid methyl esters (FAMES) were analyzed by a gas-chromatography mass-spectroscopy system (TRACE GC Ultra ISQ, Thermo Fisher Scientific) using a DB-5 column with 30 m length, 0.25 mm I.D., and 0.25 μm film thickness. Helium was used as a carrier gas. The temperature program started at 150 °C for 4 min, thereafter the temperature was ramped to 250 °C at a rate of 4 °C min^{-1} and held 5 min. The PLFAs were identified by a comparison of retention times to known standards (FAME 37 47885-U, Supelco, Inc.) and a standard bacterial acid methyl ester mixture (BAME 26 47080-U, Supelco, Inc.)⁵⁴. The contents of PLFAs (nmol g^{-1} dry soil) were quantified based on the internal standard methyl nonadecanoate (19:0). The sum of thirteen PLFAs (i15:0, a15:0, 15:0, i16:0, 16:1 ω 9, 16:1 ω 7t, i17:0, a17:0, 17:0, cy17:0, cy19:0, 18:2 ω 6 and 18:1 ω 9c) served as a measure of total microbial biomass (nmol g^{-1}). PLFAs i15:0, a15:0, 15:0, i16:0, 16:1 ω 9, 16:1 ω 7t, i17:0, a17:0, 17:0, cy17:0 and cy19:0 referred to the bacterial biomass^{55,56}. Fatty acids 18:2 ω 6 and the isomer 18:1 ω 9c were used as indicators for the fungal biomass⁵⁷. We also calculated the ratio of fungal to bacterial PLFAs (F/B).

Subsamples collected in June were used to measure soil basic properties: porosity was determined using a core method based on undisturbed soil⁵⁸; moisture was measured by an oven-dry method, and expressed on mass basis of dry soil; total organic carbon (TOC) was measured using a $\text{K}_2\text{Cr}_2\text{O}_7$ oxidation method⁵⁸; total nitrogen (TN) was measured using an element Analyzer (Elementar, Germany), and the TOC to TN ratio (C:N) was calculated; soil pH was measured with a pH meter.

Data analysis. Nematode abundances were $\ln(x + 1)$ transformed prior to statistical analysis to obtain normality of data. Using SPSS 13.0 software, all data were subjected to a one-way analysis of variance (ANOVA) with a LSD test to evaluate the reforestation age effect in both seasons. Differences at $P < 0.05$ were considered statistically significant. The relationship between nematode genera (seasonally mean data) and soil environmental variables was examined based on a redundancy analysis (RDA) using the CANOCO software. A structural equation modeling (SEM) was used to evaluate the bottom-up effect (resource control) and the connectedness of the functional groups of the nematode food web, according to documents on the interactions of the following variables^{11,14,39,59}: root biomass (R), the biomass of bacteria (B) and fungi (F), and the biomass carbon of bacterivores (BF), fungivores (FF), plant parasites (PP) and omnivores-predators (OP). The analysis was performed with AMOS 7.0 software using the 'robust' maximum likelihood estimation procedures.

References

1. Yuan, D. X. & Chai, G. H. The science of Karst environment. Chongqing Press, Chongqing species diversity and bacterial and fungal diversity in a vegetation succession on karst. *Plant Soil* **307**, 123–134 (1988).
2. Yang, X. & Hu, B. Quality characteristics of soils in Karst rocky-desertified areas with ecosystem under restoration succession—a case study of Chengjiang subwatershed, Duan county, Guangxi. *J. Ecol. Rural Environ.* **25**, 1–5 (2009).
3. He, X. Y. *et al.* Positive correlation between soil bacterial metabolic and plant species diversity and bacterial and fungal diversity in a vegetation succession on karst. *Plant Soil* **307**, 123–134 (2008).
4. Xiao, H. & Weng, Q. The impact of land use and land cover changes on land surface temperature in a Karst area of China. *J. Environ. Manag.* **85**, 245–257 (2007).

5. Yao, C. H., Jiang, Z. C. & Yuan, D. X. Vegetation effects on Karst physiognomy in southwest China. *Acta Geoscientia Sinica* **22**, 159–164 (2001).
6. Wang, S. J., Liu, Q. M. & Zhang, D. F. Karst rocky desertification in southwestern China: geomorphology, land use, impact and rehabilitation. *Land Degrad. Dev.* **15**, 115–121 (2004).
7. Wang, S. J. *et al.* How types of carbonate rock assemblages constrain the distribution of Karst rocky desertified land in Guizhou Province, PR China: phenomena and mechanisms. *Land Degrad. Dev.* **15**, 123–131 (2004).
8. Zhang, W. *et al.* The heterogeneity of soil nutrients and their influencing factors in peak-cluster depression areas of Karst region. *Sci. Agr. Sinica* **39**, 1828–1835 (2006).
9. Behera, N. & Sahani, U. Soil microbial biomass and activity in response to *Eucalyptus* plantation and natural regeneration on tropical soil. *Forest Ecol. Manag.* **174**, 1–11 (2003).
10. Yeates, G. W. Nematodes as soil indicators: functional and biodiversity aspects. *Biol. Fertil. Soils* **37**, 199–210 (2003).
11. Zhang, X. *et al.* Community composition, diversity and metabolic footprints of soil nematodes in differently-aged temperate forests. *Soil Biol. Biochem.* **80**, 118–126 (2015).
12. Háněl, L. An outline of soil nematode succession on abandoned fields in South Bohemia. *Appl. Soil Ecol.* **46**, 355–371 (2010).
13. Ferris, H. Form and function: metabolic footprints of nematodes in the soil food web. *Eur. J. of Soil Biol.* **46**, 97–104 (2010).
14. Crotty, F. V., Blackshaw, R. P. & Murray, P. J. Tracking the flow of bacterially derived ¹³C and ¹⁵N through soil faunal feeding channels. *Rapid Commun. Mass Sp.* **25**, 1503–1513 (2011).
15. Ferris, H., Sanchez-Moreno, S. & Brennan, E. B. Structure, functions and interguild relationships of the soil nematode assemblage in organic vegetable production. *Appl. Soil Ecol.* **61**, 16–25 (2012).
16. Zhao, J., Li, S., He, X., Liu, L. & Wang, K. The soil biota composition along a progressive succession of secondary vegetation in a Karst area. *Plos One* **9**, 1–9 (2014).
17. Zhao, J. *et al.* Unusual soil nematode communities on karst mountain peaks in southwest China. *Soil Biol. Biochem.* **88**, 414–419 (2015).
18. Zhao, J. *et al.* Size spectra of soil nematode assemblages under different land use types. *Soil Biol. Biochem.* **85**, 130–136 (2015).
19. Long, J., Li, J., Jiang, X., Deng, Q. & Li, Y. Effects of different recover and restoration measures on soil quality in Karst rocky desertification region. *Chin. J. Appl. Ecol.* **17**, 615–619 (2006).
20. Goralczyk, K. Nematodes in a coastal dune succession: Indicators of soil properties? *Appl. Soil Ecol.* **9**, 465–469 (1998).
21. Jiang, C., Sun, B., Li, H. & Jiang, Y. Determinants for seasonal change of nematode community composition under long-term application of organic manure in an acid soil in subtropical China. *Eur. J. Soil Biol.* **55**, 91–99 (2013).
22. Klass, J. R., Peters, D. P. C., Trohan, J. M. & Thomas, S. H. Nematodes as an indicator of plant–soil interactions associated with desertification. *Appl. Soil Ecol.* **58**, 66–77 (2012).
23. De Deyn, G. B., Raaijmakers, C. E., van Ruijven, J., Berendse, F. & van der Putten, W. H. Plant species identity and diversity effects on different trophic levels of nematodes in the soil food web. *Oikos* **106**, 576–586 (2004).
24. Wall, J. W., Skene, K. R. & Neilson, R. Nematode community and trophic structure along a sand dune succession. *Biol. Fertil. Soils* **35**, 293–301 (2002).
25. Guan, P. T., Zhang, X. K., Yu, J., Ma, N. N. & Liang, W. J. Variation of soil nematode community composition with increasing sand-fixation year of *Caragana microphylla*: Bioindication for desertification restoration. *Ecol. Eng.* **81**, 93–101 (2015).
26. Kardol, P., Bezemer, T. M., van der Wal, A. & van der Putten, W. H. Successional trajectories of soil nematode and plant communities in a chronosequence of ex-arable lands. *Biol. Conserv.* **126**, 317–327 (2005).
27. Ferris, H., Bongers, T. & de Goede, R. G. M. A framework for soil food web diagnostics, extension of the nematode faunal analysis concept. *Appl. Soil Ecol.* **18**, 13–29 (2001).
28. Zhao, J. & Neher, D. Soil energy pathways of different ecosystems using nematode trophic group analysis: a meta analysis. *Nematology* **16**, 379–385 (2014).
29. Helgason, B. L., Walley, F. L. & Germida, J. J. No-tillage soil management increases microbial biomass and alters community profiles in soil aggregates. *Appl. Soil Ecol.* **46**, 390–397 (2010).
30. Ding, X. L., Zhang, B., Zhang, X. D., Yang, X. M. & Zhang, X. P. Effects of tillage and crop rotation on soil microbial residues in a rainfed agroecosystem of northeast China. *Soil Till. Res.* **114**, 43–49 (2011).
31. Zhang, Z. Y., Zhang, X. K., Jhao, J. S., Zhang, X. P. & Liang, W. J. Tillage and rotation effects on community composition and metabolic footprints of soil nematodes in a black soil. *Eur. J. Soil Biol.* **66**, 40–48 (2015).
32. Hohberg, K. Soil nematode fauna of afforested mine sites: genera distribution, trophic structure and functional guilds. *Appl. Soil Ecol.* **22**, 113–126 (2003).
33. Deng, Q. *et al.* Soil microbial community and its interaction with soil carbon and nitrogen dynamics following afforestation in central China. *Sci. Total Environ.* **541**, 230–237 (2016).
34. Yannikos, N. *et al.* Impact of *Populus* trees on the composition of organic matter and the soil microbial community in orthic gray luvisols in Saskatchewan (Canada). *Soil Biol. Biochem.* **70**, 5–11 (2014).
35. Brzeski, M. W. Changes in the nematode fauna in the successive age classes of a Scots pine forest. *Fragm. Faun.* **38**, 339–345 (1995).
36. Ferris, H. & Manute, M. M. Structural and functional succession in the nematode fauna of a soil food web. *Appl. Soil Ecol.* **23**, 93–110 (2003).
37. Bardgett, R. D., Bowman, W. D., Kaufmann, R. & Schmidt, S. K. A temporal approach to linking aboveground and belowground ecology. *Trends Ecol. Evolut.* **20**, 634–641 (2005).
38. Lenoir, L., Persson, T., Bengtsson, J., Wallander, H. & Wir_en, A. Bottom-up or top-down control in forest soil microcosms? Effects of soil fauna on fungal biomass and C/N mineralization. *Biol. Fertil. Soils* **43**, 281–294 (2007).
39. Jonsson, M. & Wardle, D. A. Structural equation modelling reveals plant community drivers of carbon storage in boreal forest ecosystems. *Biol. Lett.* **6**, 116–119 (2010).
40. Albers, D., Schaefer, M. & Scheu, S. Incorporation of plant carbon into the soil animal food web of an arable system. *Ecology* **87**, 235–245 (2006).
41. Zhao, J., Zhang, W., Wang, K., Song, T. & Du, H. Responses of the soil nematode community to management of hybrid napiergrass: the trade-off between positive and negative effects. *Appl. Soil Ecol.* **74**, 134–144 (2014).
42. Zhang, L. Y. *Feed Analysis and Feed Quality Detection Technology* (Beijing, 2007).
43. Bongers, T. De Nematoden van Nederland in *Vormgeving en technische realisatie* (Netherlands, 1994).
44. Ahmad, W. & Jairipuri, M. S. Mononchida: the Predaceous Nematodes in *Nematology Monographs and Perspectives* Vol. 7 (ed. Brill, E. J.) 1–299 (Netherlands, 2010).
45. Yeates, G. W., Bongers, T., de Goede, R. G. M., Freckman, D. W. & Georgieva, S. S. Feeding habits in soil nematode families and genera: an outline for soil ecologists. *J. Nematol.* **25**, 315–331 (1993).
46. Andrassy, I. Die Rauminhalts- und Gewichtsbestimmung der fadenwürmer nematoda. *Acta Zool. Acad. Sci. H.* **2**, 1–15 (1956).
47. Persson, T. *et al.* Trophic structure, biomass dynamics and carbon metabolism of soil organisms in a Scots pine forest in *Structure and Function of Northern Coniferous Forests* (ed. Presson, T.) 419–459 (Stockholm, 1980).
48. Persson, T. Influence of soil animals on nitrogen mineralization in a Scots pine forest in *Proceedings of the 8th International Colloquium of Soil Zoology* (eds Lebrun, P. *et al.*) 117–126 (Belgium, 1983).
49. Shannon, C. E. A mathematical theory of communication. *Bell Syst. Tech. J.* **27**, 379–423 (1948).

50. Yeates, G. W. & Newton, P. C. D. Long-term changes in topsoil nematode populations in grazed pasture under elevated atmospheric carbon dioxide. *Biol. Fertil. Soils* **45**, 799–808 (2009).
51. Pielou, E. C. The measurement of diversity in different types of biological collections. *J. Theor. Biol.* **13**, 131–144 (1966).
52. Bongers, T. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Oecologia* **83**, 14–19 (1990).
53. Helgason, B. L., Walley, F. L. & Germida, J. J. No-till soil management increases microbial biomass and alters community profiles in soil aggregates. *Appl. Soil Ecol.* **46**, 390–397 (2010).
54. Xu, Z. *et al.* The variations in soil microbial communities, enzyme activities and their relationships with soil organic matter decomposition along the northern slope of Changbai Mountain. *Appl. Soil Ecol.* **86**, 19–29 (2014).
55. Frostegård, Å. & Bååth, E. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biol. Fertil. Soils* **22**, 59–65 (1996).
56. Zogg, G. P. *et al.* Compositional and functional shifts in microbial communities due to soil warming. *Soil Sci. Soc. Am. J.* **61**, 475–481 (1997).
57. Frostegård, Å., Bååth, E. & Tunlid, A. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biol. Biochem.* **25**, 723–730 (1993).
58. Bao, S. D. *Soil and Agricultural Chemistry Analysis* (Beijing, 2000).
59. Yeates, G. W. Effects of plants on nematode community structure. *Annu. Rev. Phytopathol.* **37**, 127–149 (1999).

Acknowledgements

This work was supported by National Science and Technology Major Project (2014ZX07101-012) and State Key Laboratory of Forest and Soil Ecology (Grant No. LFSE2015-14).

Author Contributions

X.H. and Y.L. designed the experiment; N.H., H.L., Z.T., Z.L., J.T. and G.L. carried out the field work and laboratory analysis; N.H., H.L., Z.T., Z.L., J.L. and Y.L. performed data analysis; N.H., H.L., Z.T. and Z.L. drafted the manuscript. All authors revised/reviewed the manuscript.

Additional Information

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Hu, N. *et al.* Community diversity, structure and carbon footprint of nematode food web following reforestation on degraded Karst soil. *Sci. Rep.* **6**, 28138; doi: 10.1038/srep28138 (2016).



This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>