



# Root derived C rather than root biomass contributes to the soil organic carbon sequestration in grassland soils with different fencing years

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## Abstract

**Aims** Belowground carbon (C) input is a key component of the global C cycle. However, how does fencing affect Belowground net primary production (BNPP) and the contribution of root derived C to belowground C input remains poorly studied.

**Methods** In this study, the in-growth soil cores-<sup>13</sup>C method was used to quantify net root C input. Five different plant communities of different fencing years (1 year, 5 years, 10 years, 25 years, 30 years) in the Loess Plateau of China, characterized by herbs and shrubs were investigated.

**Results** BNPP was increased with the increase of fencing years: 61.54~140.23 gC·m<sup>-2</sup>·yr<sup>-1</sup>. Root derived C in soil represented a considerable contribution to BNPP varying from 57 to 81%, and the proportion of root derived C to BNPP was decreased with the fencing years. The contribution of root derived C to soil organic carbon (SOC) was 3%~5% during one-year growth.

**Conclusions** Our results underline the fact that fencing is an effective means to improve the BNPP and plant community. Root derived C rather than root biomass contributes to the SOC sequestration in grassland soils with different fencing years. Fencing increases the contribution of root derived C to SOC mainly by increasing root C content.

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**Keywords** Root derived C · C allocation · Livestock fencing · Chines Loess Plateau · Belowground net primary production

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## Introduction

Soil has the largest reservoir of organic C in the terrestrial ecosystem. Increasing soil C sequestration is a judicious way to reduce atmospheric CO<sub>2</sub> (Lal 2004; Kell 2012) and make climate change mitigation. C from roots retained in soils is much more efficient than above-ground leaves and needles (Balesdent and Balabane 1996). So a growing interest in below-ground C fluxes and dynamics for storing increasing

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amounts of C in the soil pool to promote C sequestration (Amelung et al. 2008; De Kauwe et al. 2014; Spivak et al. 2019; Yang et al. 2019). Belowground net primary productivity (BNPP) of the ecosystem is a key component of global net primary productivity (NPP). It can be defined as all C allocated belowground by plants and not used for autotrophic respiration in a broader sense (Martinez et al. 2016). Terrestrial plants allocate more than half fixed annually through photosynthesis belowground (Grace and Rayment 2000; Gao et al. 2008). So it may represent the largest sink for gross primary production (Janssens et al. 2001). Studies have shown that root derived C is an essential component of underground C input (Amelung et al. 2008; Bradford et al. 2013; He et al. 2020), accounting for more than 50% of BNPP in grassland (Martinez et al. 2016).

Root derived C is an integral part of BNPP embedded in the soil. However, rhizodeposition belong to root derived C is difficult to be determined (Jones et al. 2004; Pausch and Kuzyakov 2018), this would lead to an underestimation of BNPP (Martinez et al. 2016). Studies have shown that the variation in  $^{13}\text{C}$  natural abundance is a sensitive approach to calculate soil C changes in soil-plant systems (Scandellari et al. 2007; Zhu et al. 2016; He et al. 2020). The in-growth soil core  $^{13}\text{C}$  technique can solve this problem effectively, so the calculation of BNPP could be more accurate. The in-growth soil core  $^{13}\text{C}$  method has also proven to be an excellent way to calculate the proportion of root derived C in BNPP (Martinez et al. 2016).

Biological factors are essential for the amount of C that accumulates in the soil (Schmidt et al. 2011). Vegetation restoration as a biological factor is one of the effective means to increase soil C sequestration and solve the greenhouse (Yang et al. 2019; Wang et al. 2020b). Fencing to exclude grazers is one of the effective ways of vegetation restoration (Golodets et al. 2010; Deng et al. 2014). Fencing has variable effects on plant community structure (Guo 2007; Wu et al. 2009) showed that species richness and diversity were lower after fencing. Deng et al. (2014) suggested that fencing simultaneously increases species richness and decreases community evenness. While fencing can significantly improve aboveground vegetation cover, vegetation biomass and productivity (Wu et al. 2009; Deng et al. 2013; Li et al. 2014). Therefore, more organic matter input to the soil leads to soil properties and SOC storage

improved (Laganire et al. 2010; Wu et al. 2010; Shi et al. 2013; Gong et al. 2017). The vegetation restoration period is an important index to reflect SOC accumulation and loss factors. Studies have shown that with the increase of vegetation restoration years, SOC increased or decreased first and then increased (An et al. 2009; Zhang et al. 2010, 2015; Deng et al. 2015). And recent research shows that vegetation restoration can influence newly assimilated C allocation in grassland communities leading to more allocation of newly assimilated C in the plant-soil system (Bai et al. 2021). Vegetation restoration has been widely used to improve the BNPP (Wang et al. 2020a). At the same time it is still unclear how BNPP changes under vegetation restoration or how the proportion of root derived C in BNPP changes. Therefore, it is of great significance to study the input of belowground C in the process of vegetation restoration under different fencing years.

We hypothesized the following: (i) fencing could increase BNPP and root derived C contributes more to BNPP than fine root C accumulation ( $\Delta C_{\text{root (fine)}}$ ) in different fencing years; (ii) the proportion of root derived C in BNPP could be decrease with the increase of fencing year due to the change of root C content. To investigate the hypotheses, we used the in-growth soil cores- $^{13}\text{C}$  method to do the experiment in a national nature reserve. The purposes of this study were to: (i) determine the effect of fencing on BNPP under different fencing years; (ii) evaluate the contribution of root derived C in belowground C input with increasing fencing years.

## Materials and methods

### Site description

Yunwu mountain (106°24'~106°28' E, 36°13'~36°19' N) is located on the Loess Plateau in China as the National Nature Reserve. Since the implementation of the enclosure policy in 1982, it has been studied as a typical grassland dominant area. *Stipa bungeana*, *Artemisia sacrorum*, and *Thymus mongolicus* have become the dominant community types with some herbs (*Heteropappus altaicus*, *Agropyron cristatum*, *Stipa grandis*, *Cleistogenes squarrosa*, *Potentilla acaulis*) and fewer shrubs. The core area in Yunwu mountain prioritized fencing (1982), and then

**Table 1** Description of five study plots characteristics

Plots	Community types	Fencing years/a	Longitude and latitude	Altitude(m)	Average coverage	Major companion species
I	<i>Cleistogenes squarrosa</i> + <i>Potentilla acaulis</i>	1	36°10'3"N,106°24'18"E	1728	40%	<i>Stellera chamaejasme</i> , <i>Euphorbia humifusa</i>
II	<i>Thymus mongolicus</i> + <i>Potentilla acaulis</i>	5	36°11'12"N,106°25'8"E	1747	60%	<i>Stipa bungeana</i> , <i>Artemisia scoparia</i>
III	<i>Stipa bungeana</i> + <i>Agropyron cristatum</i>	10	36°15'26"N,106°22'26"E	2042	70%	<i>Artemisia vestita</i> , <i>grandis</i>
IV	<i>Artemisia vestita</i> + <i>Stipa bungeana</i>	25	36°15'8"N,106°23'25"E	2058	85%	<i>Heteropappus altaicus</i>
V	<i>Stipa bungeana</i> + <i>Stipa grandis</i>	30	36°15'14"N,106°22'57"E	2053	90%	<i>Artemisia vestita</i>

the fencing area was gradually expanded. Therefore, the sample plots in the study area showed different fencing years. The soils in the study area had similar initial natural conditions before fencing (Qiu et al. 2013; Deng et al. 2014).

The grassland covers an area of about 6600 hm<sup>2</sup>, whose altitude is 1800~2100 m. The precipitation is mainly concentrated in July to September each year, and the average annual precipitation is about 425.42 mm with an arid and semi-arid climate. The soil type is dominated by gray cinnamon soil and loess soil. In April 2017, the study was performed at five plots located in different plant communities within different fencing years (Table 1): (1) 1 year ; (2) 5 years ; (3) 10 years ; (4) 25 years ; and (5) 30 years.

### Sampling scheme

Peach (*Amygdalus davidiana*) forest soil ( $\delta^{13}\text{C} \approx -11.739\%$ ) sampled 0–30 cm depth was dried at 40 °C, sieved to 2 mm well mixed to ensure homogeneity. The soil had lower nutrient content (C, N, P) than the native soil at the five study plots. The roots of the grassland ecosystem are mainly composed of fine roots ( $\leq 2$  mm in diameter). Fine roots have a higher turnover rate, and they are significant to belowground C in grassland ecosystems (Gill 2000; Freschet et al. 2013; Xia et al. 2015). So in-growth cores (2 mm mesh, 30 cm long and 5 cm in diameter) which allow fine roots to grow in were then filled with the Peach forest soil resulting in a bulk density of 1.22 g·cm<sup>-3</sup>. Cores were then inserted at the sampling plots described above.

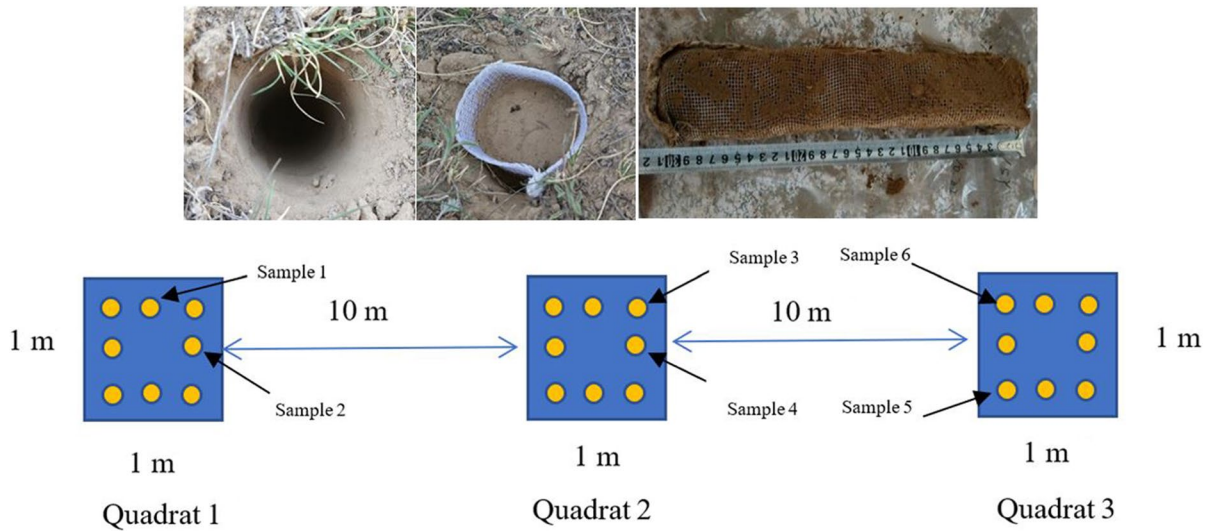
There are three quadrats (1 m×1 m) in each plot (Fig. 1). Each quadrat has eight in-growth bags, two

representative in-growth bags were selected at random. So a total of six in-growth bags were selected from 24 in-growth bags in each plot. In-growth bags were augured to a depth of 30 cm. At the top of each bag, an extra net (3–4 cm) was stitched to avoid above ground biomass input (Figure S2). The above ground biomass that fell on the bags during the research process was very small and will be cleaned regularly. The sampling depth (30 cm) was considerably sufficient to capture most of fine roots in the grassland ecosystem (Gao et al. 2008; Zanolli et al. 2013).

### Measurement and calculations

We extracted soil cores after one year, then transported them back to the laboratory. The cores were cut in half into two portions: 0–15 cm (superficial soil) and 15–30 cm (deep soil). Later fine roots were separated from the soil samples over a 1 mm sieve. Since each plot contained three quadrats, and two cores was taken out from each quadrat, hence each plot resulted into six samples which help us get the root biomass. Then all soils (two cores) in each quadrat were thoroughly mixed and the same with roots to measure the  $\delta^{13}\text{C}$ . Since each plot contains three quadrats, each sample plot produces three well-mixed samples to measure the  $\delta^{13}\text{C}$ .

Soil samples were air-dried and roots were dried at 70°C. The total C content and  $\delta^{13}\text{C}$  of roots and soil samples were analyzed using an isotope ratio mass spectrometer (IsoPrime 100 Isotope Ratio Mass Spectrometer, Germany) coupled with an elemental analyzer (Elementar ario PYRO cube, Germany). The



**Fig. 1** The layout of in-growth soil cores within each plot

SOC was determined by dichromate oxidation (Walkley and Black 1934).

C isotope values were calculated as  $\delta^{13}\text{C}$  against the international standard Vienna-Pee Dee Belemnite (V-PDB). The following equations were used:

$$\delta^{13}\text{C}(\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) * 1000$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  were molar fractions of  $^{13}\text{C}/^{12}\text{C}$  for the sample and standard, respectively.

Based on the change in  $\delta^{13}\text{C}$  values, we calculated the proportion of new soil C ( $f_{\text{NEW}}$ ) that comes from the roots and the proportion of old soil C ( $f_{\text{OLD}}$ ) that comes from the organic peach forest soil:

$$f_{\text{NEW}} = (\delta_{\text{SOIL}} - \delta_{\text{OLD}}) / (\delta_{\text{ROOT}} - \delta_{\text{OLD}})$$

$$f_{\text{OLD}} = 1 - f_{\text{NEW}}$$

$$\text{Root derived C} = (f_{\text{NEW}} * \text{SOC content} * 294.52/1000) / \pi r^2 * 10,000$$

where  $\delta_{\text{SOIL}} = \delta^{13}\text{C}$  of peach forest soil after 1 year;  $\delta_{\text{OLD}} = \delta^{13}\text{C}$  of original peach forest soil prior to insertion ( $-11.7 \pm 0.09\text{‰}$ ); and  $\delta_{\text{ROOT}} = \delta^{13}\text{C}$  of roots. Then combining the  $f_{\text{NEW}}$ , soil C content (g/kg), soil mass (294.52 g), and the radius ( $r = 2.5$  cm) of in-growth soil cores to calculate root derived C ( $\text{gC}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ ) (Cotrufo et al. 2011; Alberti et al.

2015) that was the fraction of new C derived from roots remaining in the soil core. The root derived C obtained from this calculation above contains rhizodeposition and root mortality, so it was more reliable (Martinez et al. 2016).

Using the values of root biomass which grew into the in-growth cores ( $< 2$  mm) and root C content to calculate the annual fine root C accumulation ( $\Delta\text{C}_{\text{root(fine)}}$ ):

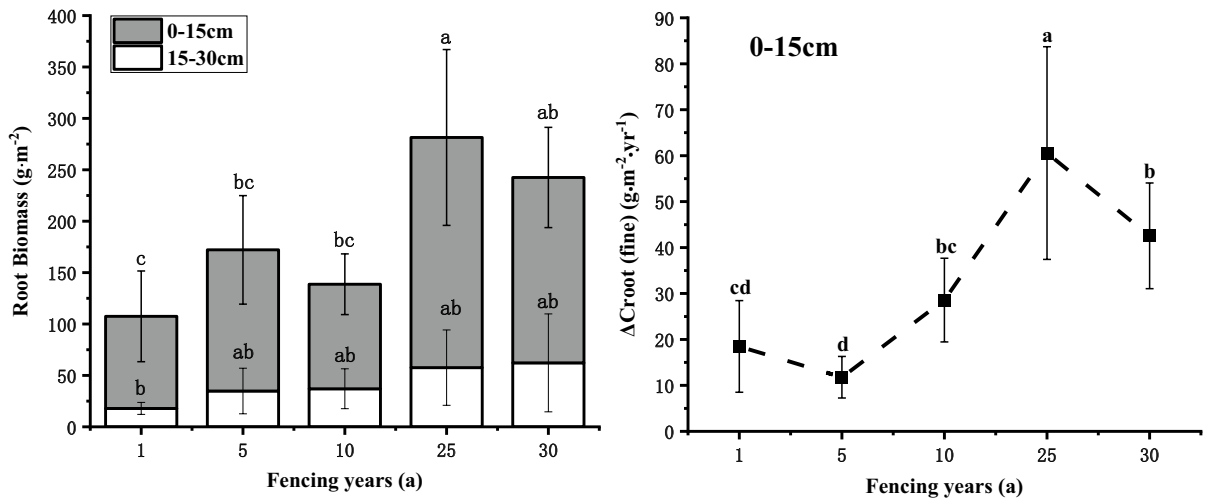
$$\Delta\text{C}_{\text{root(fine)}} = (\text{root biomass} * \text{root C content}) / \pi r^2 * 10,000$$

$$\text{BNPP} = \text{root derived C} + \Delta\text{C}_{\text{root(fine)}}$$

Belowground net primary productivity (BNPP) was calculated as the sum of  $\Delta\text{C}_{\text{root(fine)}}$  and root derived C (Martinez et al. 2016).

### Statistical analysis

All statistical analyses were performed with SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Root biomass, SOC, total carbon (TC), root  $\delta^{13}\text{C}$ ,  $\Delta\text{C}_{\text{root(fine)}}$ , root derived C and BNPP were shown as the means of 6 replicates with standard deviation and subjected to one-way ANOVA (analysis of variance) and Duncan test, with fencing time as the factor. Significant differences in treatment means were reported at the  $p < 0.05$  level.



**Fig. 2** Root biomass and C content of plant community in five study plots. Different letters indicate a significant difference among plots ( $p < 0.05$ ; Kruskal–Wallis ANOVA)

## Results

### Plant communities and root biomass

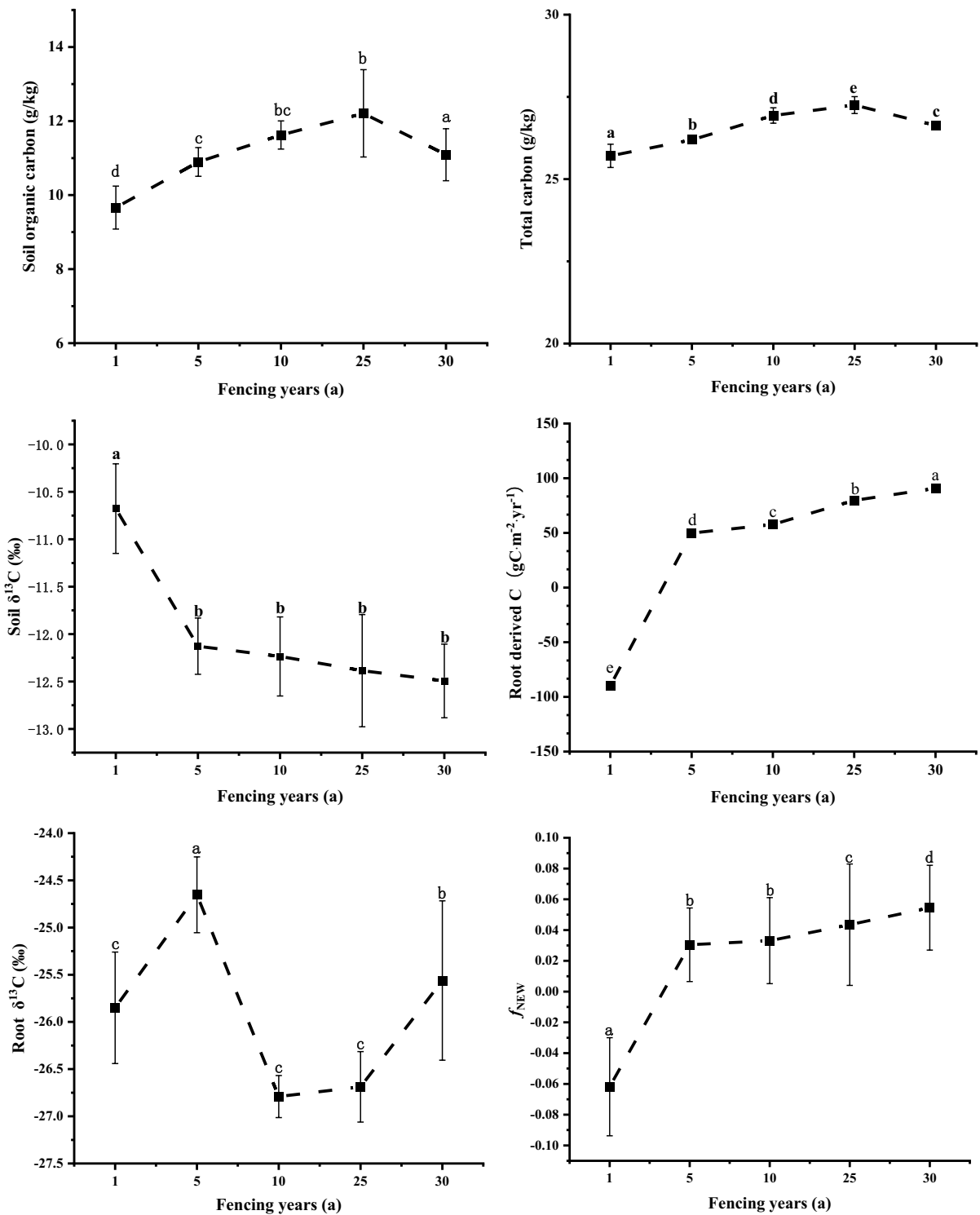
Fencing provided natural conditions for the restoration of soil and plant. Soil bulk density gradually decreased, and soil nutrients gradually increased. The plant communities succeeded from the community dominated by one or two annual plants (*Heteropappus altaicus*, *Potentilla acaulis*) to perennial plants (*Heteropappus altaicus*, *Stipa grandis*, *Aneurolepidium dasystachys*) communities with the increase of fencing years. Furthermore, the above-ground and underground biomass also increased gradually (Table S1, Table 1). It can be seen that the root biomass of the superficial layer (0–15 cm) was significantly higher than the deep layer (15–30 cm) at different fencing years (Fig. 2). At first, the root biomass showed an increasing trend, then tended to be stable with the increase of fencing years, reaching a high level ( $223.92 \text{ g}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ ) around 25 years. Since there were few roots in the deep soil layer to measure some dates, we just focused on the changes in superficial layer root productivity and soil nutrients in the next contents. During the experiment of this study, the fine roots entered in the growth bag were ordered as: 1, 2 and 3 (Figure S1). The median annual fine root C accumulation [ $\Delta C_{\text{root}(\text{fine})}$ ]

(0–15 cm) was highest ( $60.56 \pm 27 \text{ g}\cdot\text{m}^{-2}$ ) at the 25 years plot while lowest ( $11.78 \pm 2 \text{ g}\cdot\text{m}^{-2}$ ) at the 5 years plot, with a statistically significant difference between the values. Both the 30 years plot and 25 years plot had a higher root density in the superficial soil layer, whereas the 10 years plot and 5 years plot had a similar distribution in the two layers (Fig. 2).

### Belowground carbon partitioning

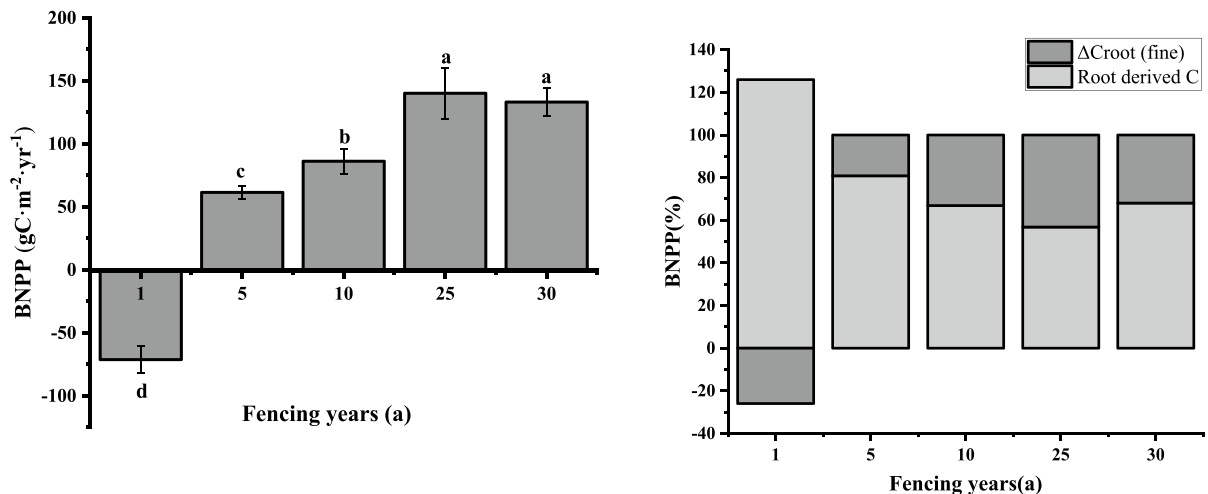
SOC, TC, root  $\delta^{13}\text{C}$ ,  $\Delta C_{\text{root}(\text{fine})}$  and the fraction of new C ( $f_{\text{NEW}}$ ) to soil showed a trend of increased first then tended to be stable with the increase of fencing years, reaching a high level around 25 years (Fig. 3). SOC content in the cores was higher than the initial SOC content (8.45 g/kg) after one year experiment, but TC was lower than the initial TC content (27.94 g/kg). The changing trend of SOC and TC was similar. At the end of twelve-month experiment, SOC content in the in-growth cores was highest (12.21 g/kg) in the 25 years plot and lowest (9.66 g/kg) in the 1 year plot. The statistically significant differences (multiple comparisons  $p$  values) were found between the 1 year plot and other plots, 25 years plot and other plots ( $p < 0.05$ ).

The statistically significant differences ( $p < 0.05$ ) were found for root  $\delta^{13}\text{C}$  value among plots instead no differences was observed for soil  $\delta^{13}\text{C}$  (according to the median values to a depth of 15 cm; Fig. 3).



**Fig. 3** Graphs showing differences among the five study plots for the various soil properties (0–15 cm) within the in-growth soil cores. Different letters indicate a significant difference among plots ( $p < 0.05$ ; Kruskal–Wallis ANOVA)





**Fig. 4** The amount of BNPP and ratio of root derived C and  $\Delta C_{\text{root (fine)}}$  to BNPP in five study plots. Different letters indicate a significant difference among plots ( $p < 0.05$ ; Kruskal–Wallis ANOVA)

The soil  $\delta^{13}\text{C}$  was found to be changed in the direction close to the root  $\delta^{13}\text{C}$  (i.e., the final soil  $\delta^{13}\text{C}$  were lower than the initial soil  $\delta^{13}\text{C}$   $-11.739\%$ ) during roots growth. The fraction of new C ( $f_{\text{NEW}}$ ) ranged between  $-0.062 \pm 0.003$  at 1 year plot and  $0.054 \pm 0.02$  at the 30 years plot, and the significant differences were observed between all plots (Fig. 3). Accordingly, root derived C (0–15 cm) ranged from  $-89.62 \pm 4$   $\text{gC}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$  (1-year plot) up to  $90.76 \pm 4$   $\text{gC}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$  (30 years plot) in the soil.

#### Belowground net primary production

The BNPP in different fencing years was ranged between:  $61.54 \sim 140.23$   $\text{gC}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$  (it was just negative in the 1 year plot:  $-71.15$   $\text{gC}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ ), and BNPP had an increasing trend with the increase of the fencing years. The fraction of C in BNPP apportioned to fine root biomass (i.e.,  $\Delta C_{\text{root (fine)}}/\text{BNPP}$ ) was similar at the 10 years (33%) and 30 years (32%) plots, while relatively lower  $\Delta C_{\text{root (fine)}}/\text{BNPP}$  was found at the 5 years (19%) plot, the highest  $\Delta C_{\text{root (fine)}}/\text{BNPP}$  was recorded for the 25 years (43%) plot (Fig. 4). As a result, a much higher proportion of BNPP was invested into root derived C (i.e. root derived C/BNPP) at the plots. The ratio of root derived C/BNPP was 81% at the 5 years plot, while in the other plots the ratio was relatively lower: 56% (25 years plot), 67% (10 years plot) and 68% (30 years plot) (Fig. 4). Overall, the C transferred belowground as

root derived C showed an increasing trend, but the proportion of root derived C had a decreasing trend with the increase of fencing years.

#### Discussion

The impact of fencing on plant communities and root biomass

Since the implementation of the vegetation restoration policy, grazing in Yunwu Mountain was significantly reduced, and grazing was utterly prohibited in the fencing area. Studies have shown that fencing can prevent animals from eating and trampling, promote plant growth, loosen soil structure, reduce bulk density and increase nutrients (Cheng et al. 2014; Vandendorj et al. 2017; Tang et al. 2019; Bai et al. 2020). The results are consistent with our research (Table S1). Moreover, fencing is beneficial to seed accumulation and rainfall infiltration in the soil, thus promoting the change of community species. Cheng et al. (2014) showed that during the 30 years of fencing in Yunwu Mountain, the degraded natural grasses succeeded mainly from the community dominated by one or two annual plants to perennial plants communities. The biomass, species richness, and coverage increased first and then kept stable with the extension of fencing time. The results are consistent with our

research (Table S1, Table 1). The species diversity (Shannon-Wiener index) varied from 0.3 to 2.9, the number of species varied from 6 to 29 species /m<sup>2</sup>, coverage varied from 22 to 90%, the density ranged between 32 and 165 plants /m<sup>2</sup> at Yunwu Mountain (Cheng et al. 2014).

This study found that root biomass decreased with the deepening of the soil layer and concentrated on the superficial soil, this was consistent with many research results (Jackson et al. 1996; Ma et al. 2008). The phenomenon was beneficial to the effective use of limited precipitation and mineral nutrient resources for vegetation in semi-arid areas (Jackson et al. 1996; Fransen et al. 1998). With the increase of soil depth, the decrease of soil temperature and structure will adversely affect root growth, so the decrease of root biomass shows the adaptability of plants to the environment.

This study found that the root biomass increased gradually and reached the maximum value around the 25 years of fencing. Because fencing measures effectively controlled the grazing and trampling of livestock on grassland vegetation, it provided natural restoration time for vegetation (Novara et al. 2013; Yunbin et al. 2014). In the early stage of fencing, the biomass increased rapidly due to the competition among species. Some studies revealed that increased plant diversity is usually accompanied by increased belowground plant biomass production (Fornara and Tilman 2010; Cong et al. 2014; Ravenek et al. 2014; Prommer et al. 2020). While in the later stage of fencing, the competition among species caused some species to withdraw from the community and dominant species tended to be in a state of minority and concentration. At the same time, soil resources can improve the survival rate of late-stage species in succession and thus change the succession of vegetation (Paterno et al. 2016; Song et al. 2019).

#### The impact of fencing on belowground carbon partitioning

SOC content in the cores was higher than the initial SOC content after one year experiment, but TC content was lower than the initial TC content. Soil C pool can be divided into organic C and inorganic C, many studies have proved that there is a negative correlation between SOC and inorganic C (Chang

et al. 2012; Shi et al. 2012; Li et al. 2016a; Zhao et al. 2016). Chang et al. (2012) found that afforestation within 20 cm of profile could increase SOC and reduce soil inorganic C in the Loess Plateau. Organic acids and other compounds secreted by the root system can also form an acidic environment in the soil, easily causing the dissolution of soil inorganic C (Li et al. 2016b). In our study, the root released a large amount of organic carbon into the soil, which result in the increase of SOC content. At the same time, the loss of soil inorganic C was much more due to the vital leaching characteristics and chemical reaction. Thus resulting in a decrease in TC content. Despite this, the trend of SOC and soil TC was consistent, and both of them showed a trend of increased first then tended to be stable with the increase of fencing years, reaching a larger value around 25 years. The reason for this phenomenon was that as the increase of fencing years, the C input into the soil by the roots also increased, however, the SOC decomposition rate was decreased (Deng 2016) as well as the TC content of the soil leaching. For the soil  $\delta^{13}\text{C}$ , aboveground plants were found to be the main influencing factors (Derrien and Amelung 2011; Pausch and Kuzyakov 2018), because soil C mainly comes from aboveground plants. In this study, the average value of plant root  $\delta^{13}\text{C}$  was  $-26.1\text{‰}$  which was lower than the average value of soil  $\delta^{13}\text{C}$  ( $-12.2\text{‰}$ ). Under the growth of plant roots, soil  $\delta^{13}\text{C}$  showed a declining trend and the lowest value was appeared in the 30-years plot. That suggest, the longer fencing time, the more C input to the soil by plant roots. At the beginning of fencing, due to the long-term shortage of soil moisture, the soil respiration was relatively increased. For one year plot, the  $\delta^{13}\text{C}$  of the soil was higher than the  $\delta^{13}\text{C}$  of the initial soil, it was because the roots had less input to the SOC and the soil was more vigorously breathing, resulting in an increase of soil  $\delta^{13}\text{C}$ . Soil  $\delta^{13}\text{C}$  was not only affected by plant  $\delta^{13}\text{C}$  but also by the metabolism process of SOC: this was mainly due to these reasons (1) plant litter is the main source of soil organic matter and soil  $\delta^{13}\text{C}$  can record the information of plant  $\delta^{13}\text{C}$  (Kuzyakov and Domanski 2000; Ge et al. 2012);(2) isotope fractionation occurs in the decomposition process of SOC. If the decomposition rate of SOC is faster, more  $^{12}\text{CO}_2$  would be released from the soil system, eventually the remaining soil would be enriched with  $^{13}\text{C}$  (Wynn 2007; Wynn and Bird 2008).



**Table 2** Correlation between roots and soil indexes

0-15 cm	SOC	Root Biomass	Root C content	BNPP	Root derived C	$\Delta C_{\text{root (fine)}}$	Fencing years
SOC	1	0.229	0.268	0.535**	0.613**	0.184	0.419*
Root Biomass		1	0.558**	0.382*	0.171	0.146	0.466**
Root C content			1	0.771**	0.444*	0.872**	0.709**
BNPP				1	0.866**	0.719**	0.947**
Root derived C					1	0.473**	0.743**
$\Delta C_{\text{root (fine)}}$						1	0.589**
Fencing years							1

\*\* $P < 0.01$  (two-tailed), \* $P < 0.05$  (two-tailed)

### Influencing factors of root derived C

With the increase of the fencing years, BNPP had an increasing trend. This was because C released by the roots into the soil (root derived C) became higher following the fencing years. Our study found that root derived C had a highly significant positive correlation with  $\Delta C_{\text{root (fine)}}$ . While there was a significant positive correlation between root derived C and root C content, there was no significant correlation between root derived C and root biomass (Table 2). This was because there was no significant difference in root biomass under different fencing years, but there was an extremely significant difference in the root C content during the 1-year growth. Lower order roots are the main part of microbial interaction with fine roots (King et al. 2021). Previous studies have shown that low order roots, which are responsible for nutrient and water uptake, have high turnover (Chen et al. 2017; Adamczyk et al. 2019). The high root turnover implies more significant C inputs into the soils (Solly et al. 2018). In this study, fine roots were composed of lower order roots. Therefore, the C content of the fine roots became an important factor affecting the belowground C input. The higher root C content was the more C released into the soil, so the root C content became the decisive factor for root derived C. Our study also found that the root growth process had more contribution to soil C than root decomposition from root derived C  $> \Delta C_{\text{root (fine)}}$ . Because  $\Delta C_{\text{root (fine)}}$  represented the total amount of C retained in roots, root derived C represented the C released into the soil during the root growth. When considering C allocation belowground pools, some interesting trends emerged: root derived C at the five plots gradually

increased, but the proportion of root derived C in BNPP decreased with the increase of fencing years. We believed the increase of root derived C was the result of rapid increase of root C content with the change in fencing years. At the same time, the rate of root C release was less than that of root C increase, leading to the gradual decrease proportion of root derived C in BNPP.

Pearson correlation analysis showed that BNPP had a positive correlation with the indexes (Table 2): Fencing years  $>$  Root derived C  $>$  Root C content  $>$   $\Delta C_{\text{root (fine)}}$   $>$  SOC  $>$  Root Biomass. This was because fencing enhanced the vegetation species, quantity and biomass, all of these together can achieve an explanation rate of 94.7% for BNPP. Comparing the five fencing years, only BNPP was negative in the plot of 1 year (Fig. 3). It was because the vegetation coverage, root biomass and increase of SOC were the lowest. While the loss of TC was the highest in one year plot (Table 3), indicating that the C released by root into the soil was less than the loss of soil C. More  $^{12}\text{CO}_2$  was released from the soil system, and the remaining soil was enriched with  $^{13}\text{C}$ , that was, soil  $\delta^{13}\text{C}$  was increased (Wynn 2007; Wynn and Bird 2008), thus resulting in a negative value of root derived C. At the same time, the absolute value of root derived C was greater than  $\Delta C_{\text{root (fine)}}$ , so the value of BNPP was negative.

Under light limitation plants tend to allocate a higher proportion of assimilated C to above-ground organs, whereas the pattern is reversed as light is no longer limiting (Litton et al. 2007). Our study showed that with the increase of vegetation coverage, the BNPP increased as well, it was because the increase of vegetation coverage can enhance photosynthetic process, which ultimately lead the boost in released C by root.

**Table 3** Characteristics of root biomass and soil carbon after one year in the soil cores

Indexes	1 year	5 years	10 years	25 years	30 years
Root biomass (g/m <sup>2</sup> )	89.55 ± 44.12	137.34 ± 52.77	101.68 ± 29.56	233.92 ± 85.55	180.29 ± 48.74
Loss of soil total carbon(g/kg)	2.05 ± 0.33	1.59 ± 0.27	1.02 ± 0.21	0.57 ± 0.31	1.24 ± 0.12
Increase of SOC(g/kg)	1.22 ± 0.47	2.45 ± 0.37	3.18 ± 0.33	3.77 ± 0.78	2.65 ± 0.50

Values are means ± standard errors (n=6)

## Conclusions

Fencing supported the improvement in the plant community and BNPP value. BNPP reached a larger value (140.23 gC·m<sup>-2</sup>·yr<sup>-1</sup>) when the plant community is stable around 30 years. The proportion of root derived C (57%~81%) to BNPP was greater than ΔC<sub>root (fine)</sub> (19%~43%), and it showed a decreasing trend with the increase of fencing years. We found that fencing which increased roots C content was a key factor affecting belowground C input.

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