

Response patterns of root sprouting and the clonal dispersal ability of *Hippophae rhamnoides* ssp. *sinensis* in relation to stubble height and its nutrient accumulation and allocation strategy

Zhongmu Li

Southwest Forestry University

Shengli Ji

People's Government of Wenfu Town, Jiaoling County

Kaihong Nie

Zhaohua Forestry Bureau of Guangyuan City

Dengjuan Tian

Southwest Forestry University

Shenghong Liu

Southwest Forestry University

Sai Lu

Southwest Forestry University

Zengyu Yao

Southwest Forestry University

Genqian Li (✉ 15825290016@163.com)

Southwest Forestry University

Article

Keywords: Stubble height, Root sprouting, Clonal dispersal, Nutrient elements, *Hippophae rhamnoides* ssp. *sinensis*

Posted Date: September 26th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3363348/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Research into the relationship between coppice regeneration and stubble height has been mostly limited to stump sprouting, but root sprouts and clonal dispersal play a more important role in the restoration of declined populations and the maintenance of population stability. We aimed to investigate the response of root sprouting (number and growth) and the clonal dispersal (root extension and branching) capacity to stubble height, unveil its nutrient accumulation and allocation strategies and determine the appropriate stubble height that is most favorable for root sprouting, clonal dispersal, and nutrient accumulation. In a 15-year-old plantation of *Hippophae rhamnoides* ssp. *sinensis* with signs of premature aging, the plants were coppiced at 0, 10, and 20 cm from the ground level, with no coppicing as the control. The results showed the following: (1) Coppicing promoted the formation and growth of root sprouts, and root extension and branching, but promotion increased and then decreased with increasing stubble height, and the optimal stubble height was 10 cm. (2) N, P, K, Ca, and Mg content and reserves all increased and then decreased with the increase in stubble height, and the clonal nutrient content, reserves, and integrated nutrient accumulation capacity were greatest at 10 cm of stubble height. (3) The coppicing increased the accumulation and allocation ratio of nutrients in leaves, vertical roots, and horizontal roots, which played a dominant role in the integrated accumulation of clonal nutrients. (4) Root sprouting and clonal dispersal ability were positively correlated with clonal nutrient content, reserves, and integrated nutrient accumulation ability. Moreover, root-sprouting ability was positively correlated with clonal dispersal ability. From these, the coppicing regulated leaf development and clonal dispersal by affecting nutrient accumulation and allocation patterns, which in turn governed the formation and growth of root sprouts. This is a continuous driving process. According to the regression equations, a stubble height of 11–13 cm was estimated to be the most favorable for root sprouting, clonal dispersal, and nutrient accumulation.

Introduction

Chinese sea buckthorn (*Hippophae rhamnoides* ssp. *sinensis*) is a multipurpose tree species widely distributed in the arid and sandy areas of northern China. It has strong capabilities in terms of nitrogen fixation, soil and water conservation, windbreak, and sand fixation. This tree is widely used in medicine, food, beverage, cosmetics, and health products¹⁻³. As a typical clonal plant, its lateral roots present a strong extending and branching ability and produces a large number of root suckers, which have the potential to fulfil "one tree makes a forest" and achieve "eternal life and immortality"⁴⁻⁸. The coupling of clonal integration and nitrogen fixation makes it more adaptable to arid and barren environments, forming a "sea buckthorn oasis" on a background of grasslands and deserts⁹⁻¹¹. Nevertheless, in recent years, drought stress has caused a large area of Chinese sea buckthorn plantation to succumb to premature aging, which is mainly manifested as retarded growth, a shortened life span, and poor regeneration, posing a great threat to the industry development and vegetation restoration of this species¹². However, the vegetation is difficult to restore by means of clearcutting and reforestation in

areas of severe desertification with arid climates and water-scarce soils¹³. Coppicing regeneration has been proven as the most ecological and economical way to solve this problem^{14,15}.

The coppicing is to cut off the main trunk of the tree at a height from the ground to promote the sprouting from stumps or roots, rejuvenating the trees and regenerating the forest¹⁶. Compared with seedling regeneration, coppicing regeneration can restore population size, structure, and function in a shorter period of time. This results in vegetation succession having a persistence niche effect, with less habitat disturbance, which is conducive to improving the adaptive capacity of the population to adverse environmental conditions and is of great importance in vegetation restoration and reconstruction. Compared with reproduction by planting after clearcutting, particularly in the context of drought, water shortage, and serious land desertification, coppicing regeneration can effectively prevent secondary desertification and recurrence of soil erosion¹⁷⁻²⁰. Coppicing can release apical dominance and directly or indirectly regulate the synthesis, accumulation and allocation of hormones and nutrients^{21,22}. However, the sprouting effectiveness of coppiced tree is affected by many factors²³, among which the stubble height is one of the controllable factors. Two questions therefore remain: Do different stubble heights and different organ components of the coppiced tree contribute differently to the nutrient accumulation? If so, is there a causal relationship between these differences and the sprouting capacity? Understanding such questions is of theoretical and practical importance for exploring the nutrient accumulation and allocation strategy of coppicing regeneration and for determining the appropriate stubble height.

Forest regeneration is an important ecological process in forest community succession and vegetation ecological restoration^{24,25}. Zhu et al.²⁶ proposed six hypotheses regarding the mechanisms of forest regeneration. The nutrient hypothesis suggests that tree sprouting capacity and sprout growth are related to the root system and soil nutrient storage of the parent tree. The resource allocation hypothesis suggests that the sprouting regeneration capacity after the loss of above-ground parts depends on the resource storage of the above-ground and below-ground organs of the parent tree. However, few studies have reported the relationship of nutrient accumulation and allocation pattern with disturbance intensity (e.g., stubble height). At the same time, there are different mechanisms for tree regeneration after coppicing. Some species are regenerated by sprouting from stumps, while others by sprouting from stumps and roots^{25,27,28}. Research into the relationship between coppice regeneration and stubble height has been mostly limited to stump sprouting^{19,27}. In fact, the root sprouting is more important for recovering population size and long-term maintaining population stability than stump sprouting²⁹. On the one hand, the root sucker has the potential to survive independently and reproduce asexually, which plays a decisive role in the population succession trend^{30,31}. On the other hand, root extension (clonal proliferation) and branching are prerequisites for the formation and growth of root sucker, and they also represent the main approach for population dispersal of clonal plants^{29,32,33}. Therefore, studying the effects of stubble height on root sprouting and clonal dispersal would be helpful to understand the similarities and differences of mechanisms between the root sprouting and stump sprouting, and it could also provide case support for the theory of forest regeneration by coppicing.

This study took Chinese sea buckthorn as the experimental plant and stubble height as the experimental factor to investigate the following: (i) the response patterns of root sprout (number and growth) and the clonal dispersal (root extension and branching) capacity to the stubble height; (ii) the response patterns of the nutrient accumulation capacity (content and reserves) and allocation pattern of clones and their components to stubble height; and (iii) the relationship of root sprouting and clonal dispersal with nutrient accumulation and their allocation pattern. The authors aim to reveal the nutritional mechanisms involved in the regulation of root sprouting and clonal dispersal ability of coppiced Chinese sea buckthorn and provide a theoretical basis for the selection of a suitable stubble height in its plantations.

Materials and methods

Experimental area

The experimental area was in Dingbian County, Shaanxi Province, China, in the transitional zone between the Mu Us Sands and the Loess Plateau (107°15'~108°22'E, 36°49'~37°53'N, altitude of 1303 ~ 1907m). It is a mid-temperate semi-arid continental monsoon climate with low precipitation (annual average 316 mm), high evaporation (annual average 2490 mm), strong and frequent wind and sandstorms, an annual average temperature of 7.9 °C, an annual maximum temperature of 37.7 °C, and an annual minimum temperature of - 29.4 °C. The soils are mainly aeolian sandy soil and yellow sandy soil, with a poor nutrient content, and poor water and fertilizer retention capacity. The zonal vegetation is arid steppe and desert steppe, with xerophytes, psammophytes, halophytes, and mesophytic meadow plants, among which herbaceous plants mainly include *Agriophyllum squarrosum*, *Psammochloa villosa*, *Agropyron cristatum*, etc.

The experiment commenced in 2016 in a plantation on a loess hill named Weiliang Hill at Baiwanzi Town, which was established in 2001 with a planting spacing 1.0 m × 1.5 m. The soil was loessial soil covered with a layer of sand. The stand contained the initial planted maternal plants of Chinese sea buckthorn and their root suckers, with a canopy density of 0.8. Before the coppicing, the trees were uniform in growth with some premature aging characteristics, including death of some branches, leaves, and even trunks, and reduction in growth rate and reproduction capacity¹².

Materials

Chinese sea buckthorn is an endemic species (subspecies) of China. It is a small tree or shrub of the genus *Hippophae* L. in the family Elaeagnaceae Juss. It is widely distributed in the arid and semi-arid regions of northern China, occupying 85% of the total area of *Hippophae* L. in China³⁴. Its root system can be divided into vertical and horizontal roots. Vertical roots grow vertically or inclined downward, while horizontal roots extend horizontally with the topography at a certain depth of soil from the surface^{7,8}. After the mother plant (the original parent for clonal propagation) is settled (or planted), the population spreads by the long-distance extension of horizontal roots, multiple branching, and root suckers. Each mother plant and its ramets (root suckers) are connected by horizontal roots to form a clone.

Consequently, the stand consisted of multiple clones (Fig. 1). Clones, as an ecological and physiological unit, can respond integrally to environmental resource levels or heterogeneity, and their plasticity is mainly expressed at the levels of clonal components and ramet components³⁵. Among them, the clonal components can be divided into the mother plant, daughter ramet, and root system (including horizontal and vertical roots); the ramet (including mother plant and daughter ramet) components can be divided into the leaves, branches, and trunk. The vertical roots and horizontal roots are divided into primary, secondary, tertiary, and quaternary levels; the primary roots are from the mother ramet, the secondary roots are from the primary roots, and so on.

Experiment layout

The stubble height was set at 3 gradients of 0, 10.0, and 20.0 cm, according to the regression experimental design requirements and pre-experiment results, with no coppicing as the control, denoted by 1, 2, 3, and CK, respectively. The field layout was a combination of a randomized block and Latin square design, with 4 replications; however, at least 8 replications were statistically available. In this case, each treatment (plot) consisted of 10 clones (each consisting of a planted mother ramet and its root system and root tillers). The mother ramet of each clone was coppiced, and its stubble was marked and protected by applying paint, keeping the root-tillers (daughter ramets) connected to the mother ramet. The experiment commenced in April 2016 and was followed up on the 25th of each month until October, and so on for three consecutive years. The final investigation was conducted in 2018.

Survey methods

Each plot was divided into 10 quadrats of 1.0 m×1.5 m (initial plant spacing) centered on the mother plant, regarded as one clone. The parameters regarding root sprouting ability (quantity and growth) and clonal dispersal ability (root extension and branching) were measured. The root sprouting ability was represented by the number, height, and ground diameter of root suckers. The clonal dispersal ability was represented by the diameter, length, and number of first-order horizontal roots and total number of horizontal roots at all levels. The horizontal root extension ability was represented by the diameter and length of the first-order horizontal root. The branching intensity was represented by the number of the first-order horizontal root and total number of horizontal roots. After the determination of the parameters of the root sprouting ability, the quadrat with the closest sprouting ability to the average value of each plot was selected as the average standard clone. Then, all the underground components of the average standard clone were dug out for the measurement of clonal dispersal capacity.

Measurement of phenotypic traits

Seven phenotypic traits that are genetically relatively stable and easy to obtain and measure were selected to characterize the clone, namely the number of root sprouts (NRS), height of root sprouts (HRS), ground diameter of root sprouts (GDRS), length of the first-order horizontal root (1ST-LHR), diameter of the first-order horizontal root (1ST-DHR), number of first-order horizontal roots (1ST-NHR) and total number of horizontal roots (TNHR). The number of root sprouts were counted one by one. The height of the root

sprout and length of the first-order horizontal root were measured with a steel tapeline (accuracy of 0.01 m). The ground diameter of root sprouts and the diameter of the first-order horizontal root were measured with a vernier caliper (accuracy of 0.01 mm). The measurements were repeated 3 times and the mean was recorded. Since the first-order horizontal roots usually extend beyond the quadrat, the length of the horizontal roots within the quadrat was taken as the reference index of extension ability and the diameter as the main index.

Determination of nutrient elements

The above-ground components of the average standard clones were divided into leaves, branches, and trunks, and the below-ground components were divided into horizontal and vertical roots. After the fresh weight was determined, the biomass was sampled and brought to the laboratory for the determination of dry biomass and nutrient content. The dry biomass was determined by oven drying at 105 °C to constant weigh. The dried sample was ground and sieved for nutrient content determination. N content was determined using the Kjeldahl method after H₂SO₄-H₂O₂ digestion, P content using the molybdenum–antimony anti-colorimetric method, K content using the flame photometer method, and Ca and Mg contents using the atomic absorption photometer method^{36–38}. Finally, the nutrient reserves were calculated according to the nutrient content and the dry biomass of corresponding components (leaves, branches, trunks, vertical roots, horizontal roots), and the distribution proportion of different nutrient elements in each component (as a percentage of the total amount) was calculated. Nutrient reserve was calculated as:

$$M = T \times B \quad (1)$$

where M is nutrient reserve, T is nutrient content in dry biomass (g·kg⁻¹), and B is Dry weight biomass(kg).

Data analysis

Duncan multiple comparison was used to state statistical difference of the reported data between treatments. The causality of root sprouting and clonal dispersal relating to nutrient content and reserve was assessed by correlation analysis. The contributions of different components, nutrient elements, and stubble height to the integrated nutrient accumulation in clone were obtained by principal component analysis (PCA), and their relative importance was ranked according to the corresponding principal component score³⁹.

We established m principal component equations for each nutrient element, and the equation expression was as follows:

$$F_i = \sum_{j=1}^m e_{ij} a_i \quad (i = 1, 2, 3, \dots, m; j = 1, 2, 3, \dots, m) \quad (2)$$

where F_i is the score of the i -th principal component equation; e_{ij} is the eigenvector; a_j is the factor loading of the i -th index of the nutrient element. Based on the data and Eq. (2), m principal component scores were calculated for each nutrient element.

The scores of the m principal components were weighted and summed to obtain the score of the principal component, which was the integrated index F of nutrient element accumulation. The formula is as follows:

$$F = \alpha_1 F_1 + \alpha_2 F_2 + \alpha_3 F_3 + \dots + \alpha_m F_m \quad (3)$$

where F is the integrated index of nutrient accumulation; α is the weighting factor, which is the percentage of the contribution rate of each principal component to the cumulative contribution rate of m principal components; $F_1 - F_m$ is the score of m principal components for each nutrient element.

The Duncan multiple comparison and the principal component analysis were performed using SPSS17.0. The regression analysis, equation construction and graphing were carried out using Excel 2016. The correlation analysis and graphing were conducted using Origin 2021.

Ethical approval

We confirm that all the experimental research and field studies on plants (either cultivated or wild), including the collection of plant material, complied with relevant institutional, national, and international guidelines and legislation. All of the material is owned by the authors and/or no permissions are required.

Results

Response of root sprouting and clonal dispersal ability to stubble height

According to Fig. 2, root sprouts appeared from the coppiced trees but not from CK. The number, height, and ground diameter of root sprouts were, in descending order, 10 cm, 20 cm, 0 cm, and there were differences among different stubble heights. The length of the first-order horizontal root, the diameter of the first-order horizontal root, number of first-order horizontal roots and the total number of horizontal roots were, in descending order, 10 cm, 20 cm, 0 cm, CK. Thus, the coppicing improved the root sprouting and clonal dispersal ability, with the best of 10 cm coppicing.

Response of biomass to stubble height

According to Fig. 3A, the coppicing changed the biomass of each component. The biomass from heavy to light is 10 cm, 20 cm, CK, 0 cm for leaf, CK, 10 cm, 20 cm, 0 cm for branch, 10 cm, CK, 20 cm, 0 cm for trunk, 10 cm, 20 cm, CK, 0 cm for vertical root, and 10 cm, 20 cm, 0 cm, CK for horizontal root. The difference between treatments was statistically significant. In conclusion, the biomass increased first and then decreased with the increase of stubble height, and 10cm of stubble height was the most beneficial to the maintenance of biomass.

Response of nutrients to stubble height

Response of nutrient content to stubble height

It can be seen from Fig. 3B that the coppicing significantly increased the clone's N, P, and K content but significantly decreased its Ca and Mg content. Moreover, the nutrient content increased and then decreased with the increase in stubble height.

Response of nutrient reserves to stubble height

As is shown in Fig. 4, the nutrient element reserves of the clone and its components either increased or decreased after the coppicing. However, all showed a trend of first increasing and then decreasing with the increase in stubble height: the minimum, maximum and intermediate were for the coppicing height of 0, 10, and 20 cm, respectively. The nutrient element reserves of 0 cm stubble were not more than the control. For the reserves in the 10 cm stubble clone, the nutrient elements in leaves, vertical roots, and horizontal roots were significantly higher than the CK; nitrogen, phosphorus, and potassium in the trunks and branches were higher than the CK or not significantly different from the CK; however, calcium and magnesium were significantly lower than the CK. For the reserves of 20 cm coppicing, phosphorus was higher than the CK; the nitrogen, potassium, and magnesium were not significantly different from the CK; however, the calcium reserves were significantly lower than the CK. The results indicated that 10 cm coppicing was the most beneficial for the accumulation of nutrient elements.

Response of nutrient allocation to stubble height

As is shown in Fig. 5, the nutrient reserves accounted for 60%-80% in above-ground components and 20%-40% in below-ground components for the clones without coppicing (CK), while those accounted for 30%-60% and 40%-70% in above-ground and below-ground components, respectively, for the clones with coppicing, indicating that the coppicing made more nutrients allocated to below-ground components. Among above-ground components, the allocation increased to leaves while decreased to branches and trunks; Among below-ground components, it increased significantly to both horizontal and vertical roots. This indicates that coppicing changed the nutrient allocation pattern, increasing the allocation to leaves, horizontal roots, and vertical roots, and reducing the allocation to trunks and branches.

Response of root sprouting and clonal dispersal capacity to nutrient elements

Response of root sprouting and clonal dispersal capacity to nutrient content and reserves

As is shown in Fig. 6, the number of root sprouts and their growth, horizontal root extension capacity (diameter of first-order horizontal root), and branching intensity were significantly or highly significantly positively correlated with nutrient content and reserves. The number of root sprouts and their growth were significantly or highly significantly correlated with horizontal root extension capacity and branching intensity. This indicated that the root sprouting and clonal dispersal ability increased with the increase in

nutrient content and reserves, and the number of root sprouts and their growth would increase with the increase in root extension and branching.

Response of root sprouting and clonal dispersal capacity to combined effect of nutrients

To understand the contribution of different components, different nutrient elements, and different stubble heights to the integrated accumulation of clonal nutrient elements, PCA was used to simplify the data dimensionalities and the complexity. Two principal components were extracted from the nutrient elements in different clone components (Fig. 7A), with a cumulative loading of 97.3%. The first principal component (contribution rate of 64.5%) was mainly based on the nutrient elements in the resource-acquiring organs, including N, P, K, Ca, and Mg in leaves, vertical roots, and horizontal roots. The second principal component (contribution rate of 32.8%) was mainly based on the nutrient elements in the resource-transporting organs, including N, P, K, Ca, and Mg in branches and trunks. According to the principal component scores, the contribution of different clone components to the integrated accumulation of nutrients decreased in the order of leaves, vertical roots, horizontal roots, branches, and the trunk. Two principal components were extracted from the PCA of different nutrients (Fig. 7B), with a cumulative loading of 99.9%. The first principal component (contribution rate of 52.4%) was mainly characterized by massive nutrients, including N, P, and K. The second principal component (47.5% contribution) was mainly characterized by trace elements, including Ca and Mg. The contribution of different nutrients to the integrated accumulation of nutrients in the clone decreased in the order of P, N, K, Mg, and Ca, according to the order of the principal component scores.

Discussion

Coppicing is a common and important measure in forestry practices^{40,41}. The results of this study showed that the nutrient content and reserve in the clone of Chinese sea buckthorn increased and then decreased with the increase in stubble height. The number and growth of root sprouts, horizontal root extension, and branching capacity were positively correlated with the nutrient content and reserves, so they also tended to increase first and then decrease. The results of the PCA also showed that the 10 cm stubble clone had the strongest integrated capacity of nutrient accumulation. These results indicate that this tree has a certain limit of tolerance to coppicing disturbance, and only at a suitable stubble height is the nutrient accumulation capacity restored and brought into play; too low a stubble height (too strong disturbance) or too high a stubble height (too weak disturbance) will inhibit or impair the nutrient accumulation capacity, which is not conducive to the development of the nutrient accumulation potential. The reason for this is that the coppicing removes senescent tissue, restores growth vigor, and stimulates the plant to grow complementarily^{42,43}. Removal of senescent tissue increases nutrient concentration in the body, thus activating lateral bud meristematic tissue^{44,45}. The studies by Bai et al.⁴⁶ and Zhang et al.⁴⁷ on the sprouting of Chinese sea buckthorn showed that the endogenous hormone (IAA, GA₃, ZR ABA) content and ratio play a decisive regulatory role in the sprouting process. The study of sea buckthorn by Shah et al.⁷ showed that highest production of shoots from roots under *in vitro* conditions

occurred in W4 medium (WPM medium with added IAA, benzyl adenine and gibberellic acid) after pre-treatment with high P in the presence of IAA while horizontal root production in WPM (Woody plant medium) was stimulated by IAA in low and middle P. These results indicate that hormone and nutrient elements interact during the root sprouting process of sea buckthorn. However, Bond¹⁷ and Maschinski⁴⁸ pointed out that plants' response extent is related to the intensity of subjected disturbance. It has also been shown that when plants are disturbed, the nutrients and energy in their bodies change in response to the degree of disturbance, which is the way plants adapt to environmental changes through physiological regulation⁴⁹. The same trend of increasing first and then decreasing with the increase in stubble height has also been reported on the biomass accumulation, non-structural carbohydrate content, and reserves of the clones and their components^{50,51}. This variation pattern is in accordance with the law of Shelford's tolerance, that is, the tolerance ability of nutrient element accumulation to the coppicing disturbance first increased and then decreased with the increase in stubble height; only the appropriate height of stubble was conducive to the development of nutrient element accumulation potential. Obviously, based on the "nutrient hypothesis" and "resource allocation hypothesis" of forest sprouting, this study further clarified the response pattern of nutrient accumulation capacity of Chinese sea buckthorn to stubble height (disturbance intensity), and confirmed the positive correlation of root sprouting and clonal dispersal to nutrient accumulation capacity.

Despite the above, not all the components play the same role in the nutrient accumulation. Specifically, coppicing improved nutrient accumulation and allocation in leaves, vertical roots, and horizontal roots. Leaves are the main organs of green plants for photosynthesis. Increasing nutrient accumulation and allocation in leaves can promote growth and development of leaves, which is conducive to the rapid recovery of photosynthetic physiological functions and carbohydrate accumulation, thus promoting sprouting and sprout growth^{52,53}. The horizontal root of Chinese sea buckthorn has multiple functions such as nitrogen fixation, clone reproduction, and resource storage and transportation, while the main function of the vertical root is underground resource acquisition⁵⁴. Obviously, increasing the accumulation and allocation of nutrient elements in the root system is conducive to promoting the growth and development of the root system, thus increasing the range and capacity for resource acquisition from soil. In particular, the increase in the content, reserves, and allocation of nutrient elements in horizontal roots can promote their extension and branching, which is conducive to root sucker reproduction, resource storage, and the resource sharing among the interconnected ramets, thus accelerating population recovery and dispersal^{6,51,54}. Bellingham and Sparrow²⁴ also noted that when plants are disturbed, they will devote more resources to regeneration and allocate more resources to the organs with the most active physiological functions. Coppicing deprives plants of most of their above-ground biomass, while sprout regeneration requires support by meristem and nutrients⁵⁵⁻⁵⁷. At the same time, the plant's stored nutrients are consumed during the sprouting process after the stem is cut off, indicating that the sprout growth also consumes nutrients⁵⁸. Compared with the "nutrient hypothesis" and "resource allocation hypothesis", this study clarify that different components have different nutrient accumulation capacity and thus show different importance in the recovery or development of root sprouting and clonal dispersal, that is, the leaves and roots are more important than other components.

The number of root sprouts and growth of coppiced Chinese sea buckthorn were positively correlated with horizontal root extension and branching ability, indicating that clonal dispersal is not only the material basis for sprouting from roots, but also one of the main sources of energy and material for the sprouting and growth of root sprouts^{50,51}. Combining the above findings, it is clear that there is a continuous driving process in terms of stubble height affecting the nutrient accumulation and allocation pattern, which in turn affects the dispersal and branching ability of roots, and ultimately affects the formation and growth of root sprouts (Fig. 8). In other words, coppicing regulates the sprouting, sprout growth and clonal dispersal by changing the nutrient content and reserves in each component and its allocation pattern.

Another important point to note is that the root sprouts of sea buckthorn originate from lateral roots and can form their own vertical roots, thus having the potential to survive independently. After the death of the mother ramet, its cloned offspring can continue to reproduce asexually, thus giving the plant the potential for "immortality"^{7,8,50,54}. Depending on its strong extension and branching ability of horizontal roots, this species can spread from a slack to slope or even top of dune, from a river valley (lower wetland) to surrounding migratory sandy land, and from a bottom to slope of gully. Consequently, vegetation cover can be obtained in the section difficult for afforestation. Unlike root sprouts, the stump sprouts from the same stump can self-thin, and "replacing removed trunk with stump sprout" is also a pending question^{19,27}. Therefore, root sprouting is more important for forest regeneration through coppicing, and the study on the root-sprouting pattern and mechanism will be beneficial to the improvement of forest regeneration theory.

Conclusion

The nutrient accumulation capacity (content and reserves), root sprouting capacity (number and growth), and clonal dispersal capacity (root extension and branching) of Chinese sea buckthorn showed an increasing and then decreasing trend with increasing stubble height. Under the conditions of stubble height in 0, 10 and 20 cm, the nutrient accumulation, root sprouting and clonal dispersal of Chinese sea buckthorn in 10 cm were optimal. The results of this study revealed the response of root sprouting, clonal dispersal, and nutrient accumulation ability of Chinese sea buckthorn to stubble height, and also reflected the importance of the appropriate stubble height.

On the other hand, Chinese sea buckthorn responded to the stubble height through the regulation of nutrient element distribution pattern. Coppicing increases the allocation ratio of nutrient in leaves, vertical roots, and horizontal roots, while decreasing the allocation ratio of nutrient in trunks and branches. According to the score of principal component analysis, the contribution of leaves, vertical roots, horizontal roots, branches and trunks to the comprehensive accumulation of nutrients decreased successively. The results of this study revealed the nutrient allocation strategy of Chinese sea buckthorn after coppicing, and also reflected the differences in the role of different components in the process of nutrient comprehensive accumulation.

The results also showed that, the root sprouting and clonal dispersal capacity were positively correlated with nutrient accumulation capacity, and the root sprouting capacity was positively correlated with clonal dispersal capacity. Combined with the above research results, it can be concluded that coppicing affects the accumulation ability and allocation pattern of nutrient, which in turn affects the dispersal and branching ability of roots, and ultimately effects the formation and growth of root sprouts, which is a continuous driving process. The results of this study revealed the nutritional mechanism of root sprouting and clonal dispersal.

In summary, on the basis of revealing the response law of root sprouting, clonal dispersal and nutrient accumulation ability of Chinese sea buckthorn to stubble height, this study clarified the nutrient accumulation and allocation strategy of Chinese sea buckthorn after coppicing, as well as the causal relationship between nutrient accumulation, allocation pattern, root sprouting and clonal dispersal. This study provided a new case support for the improvement of the nutrition hypothesis and resource allocation hypothesis of forest regeneration, and also provided a theoretical basis for the choice of suitable stubble height of Chinese sea buckthorn plantation.

Declarations

Acknowledgements

This work was funded by the National Natural Science Foundation of China (31570609, 31070551, 30371193).

Author contributions

G.L. and Z.Y. designed the research; K.N. and S.J. conducted the research; S.L. and S.H.L. collected the data; Z.L. and D.T. analyzed the data; Z.L. wrote the paper. All authors have read and agreed to the published version of the manuscript.

Data availability

The raw data supporting the conclusions of this article will be made available by the correspondence author without undue reservation. (G.L.)

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Additional information

Correspondence and requests for materials should be addressed to G.L.

Ethics approval and consent to participate

Sample and phenotypic datas were collected from 15 years old trees at the transitional zone between the Mu Us Sands and the Loess Plateau (107°15'-108°22'E, 36°49'-37°53'N) The transitional zone is a public institution. Sample and data collection were approved by the management of the transitional zone.

References

1. Kanayama, Y. *et al.* Research progress on the medicinal and nutritional properties of sea buckthorn (*Hippophae rhamnoides*) – a review. *The Journal of Horticultural Science and Biotechnology* 87, 203–210, doi:10.1080/14620316.2012.11512853 (2015).
2. Guan, T. T., Cenkowski, S. & Hydamaka, A. Effect of Drying on the Nutraceutical Quality of Sea Buckthorn (*Hippophae rhamnoides* L. ssp. *sinensis*) Leaves. *Journal of Food Science* 70, E514-E518, doi:10.1111/j.1365-2621.2005.tb08312.x (2005).
3. Hussain, A. *et al.* A supernatural multipurpose plant sea buckthorn (*Hippophae rhamnoides* L.): An updated overview of its folk traditional uses, phytochemical profile and biological activities. *Journal of Berry Research*, 1–46, doi:10.3233/JBR-220055 (2023).
4. Cao, Z. *et al.* Modular growth and clonal propagation of *Hippophae rhamnoides* subsp. *sinensis* in response to irrigation intensity. *Journal of Forestry Research* 27, 1019–1028, doi:10.1007/s11676-016-0236-z (2016).
5. Price, E. A. & Marshall, C. Clonal plants and environmental heterogeneity–An introduction to the proceedings. *Plant Ecology* 141, 3–7, doi:10.1023/A:1009838300691 (1999).
6. Takahashi, M. K., Horner, L. M., Kubota, T., Keller, N. A. & Abrahamson, W. G. Extensive clonal spread and extreme longevity in saw palmetto, a foundation clonal plant. *Molecular ecology* 20, 3730–3742, doi:10.1111/j.1365-294X.2011.05212.x (2011).
7. Shah, S. R. U., Plaksina, T., Sriskandarajah, S. & Lundquist, P.-O. Shoot organogenesis from roots of seabuckthorn (*Hippophaë rhamnoides* L.): structure, initiation and effects of phosphorus and auxin. *Trees* 29, 1989–2001, doi:10.1007/s00468-015-1278-0 (2015).
8. Shah, S. R. U., Agback, P. & Lundquist, P.-O. Root morphology and cluster root formation by seabuckthorn (*Hippophaë rhamnoides* L.) in response to nitrogen, phosphorus and iron deficiency. *Plant and Soil* 397, 75–91, doi:10.1007/s11104-015-2598-y (2015).
9. Wang, J.-C., Shi, X., Yin, L.-K. & Zhang, D.-Y. Role of clonal integration in life strategy of sandy dune plant, *Eremosparton songoricum* (Litv.) Vass (Fabaceae): experimental approach. *Polish Journal of Ecology* 3, doi:10.1149/1.2425539 (2011).
10. Yan, X., Wang, H., Wang, Q. & Rudstam, L. G. Risk spreading, habitat selection and division of biomass in a submerged clonal plant: Responses to heterogeneous copper pollution. *Environmental Pollution* 174, 114–120, doi:10.1016/j.envpol.2012.10.013 (2013).
11. Yu, F., Dong, M. & Krüsi, B. Clonal integration helps *Psammochloa villosa* survive sand burial in an inland dune. *New Phytologist* 162, 697–704, doi:10.1111/j.1469-8137.2004.01073.x (2004).

12. Zhang, Z., Jiang, Z., Li, T., Xiao, Z. & Li, G. Causes and features of *Hippophae rhamnoides* ssp. *sinensis* plantation premature aging in Mu Us Sandland. *Journal of Northwest Forestry University* 31, 1 ~ 6, doi:10.3969/j.issn.1001-7461.2016.06.01 (2016).
13. Feng, T. *et al.* Long-term effects of vegetation restoration on hydrological regulation functions and the implications to afforestation on the Loess Plateau. *Agricultural and Forest Meteorology* 330, 109313, doi:10.1016/j.agrformet.2023.109313 (2023).
14. Wang, H., Qi, W., Guo, Y. & Xu, Y. Effects of stumping on fine root architecture, growth, and physiology of *Hippophae rhamnoides*. *PeerJ* 11, e14978, doi:10.7717/PEERJ.14978 (2023).
15. Liu, L., Guo, Y., Liu, X., Yao, Y. & Qi, W. Coordinated variation in root and leaf functional traits of *Hippophae rhamnoides* treated at different stump heights in feldspathic sandstone areas of Inner Mongolia. *Frontiers in Plant Science* 14, doi:10.3389/FPLS.2023.1104632 (2023).
16. Luoga, E. J., Witkowski, E. & Balkwill, K. Regeneration by coppicing (resprouting) of miombo (African savanna) trees in relation to land use. *Forest Ecology and Management* 189, 23–35, doi:10.1016/j.foreco.2003.02.001 (2004).
17. J.Bond, W. & J.Midgley, J. Ecology of sprouting in woody plants:the persistence niche. *Trends in Ecology & Evolution* 16, doi:10.1016/S0169-5347(00)02033-4 (2001).
18. Rydberg, D. Initial sprouting, growth and mortality of European aspen and birch after selective coppicing in central Sweden. *Forest Ecology and Management* 130, 27–35, doi:10.1016/S0378-1127(99)00187-5 (2000).
19. Keyser, T. L. & Zarnoch, S. J. Stump sprout dynamics in response to reductions in stand density for nine upland hardwood species in the southern Appalachian Mountains. *Forest Ecology and Management* 319, 29–35, doi:10.1016/j.foreco.2014.01.045 (2014).
20. Moreira, B., Tormo, J. & Pausas, J. G. To resprout or not to resprout: factors driving intraspecific variability in resprouting. *Oikos* 121, 1577–1584, doi:10.1111/j.1600-0706.2011.20258.x (2012).
21. Han, X., Liang, X., Ma, S., Wang, Y. & Lu, X. Depletion of stem non-structural carbohydrates supports release of lateral branches from apical control in *Quercus mongolica* seedlings. *Trees* 36, 1573–1584, doi:10.1007/s00468-022-02313-0 (2022).
22. Beveridge, C. A., Rameau, C. & Wijerathna-Yapa, A. Learnings from a century of apical dominance research. *Journal of Experimental Botany*, erad137, doi:10.1093/jxb/erad137 (2023).
23. Xu, L. *et al.* Trampling, defoliation and physiological integration affect growth, morphological and mechanical properties of a root-suckering clonal tree. *Annals of Botany* 109, 1001–1008, doi:10.1093/aob/mcs006 (2012).
24. Bellingham, P. J. & Sparrow, A. D. Resprouting as a life history strategy in woody plant communities. *Oikos* 89, doi:10.1034/j.1600-0706.2000.890224.x (2000).
25. Mostacedo, B., Putz, F. E., Fredericksen, T. S., Villca, A. & Palacios, T. Contributions of root and stump sprouts to natural regeneration of a logged tropical dry forest in Bolivia. *Forest Ecology and Management* 258, 978–985, doi:10.1016/j.foreco.2008.09.059 (2009).

26. Zhu, W., Wang, J., Luo, C. & Duan, X. Progresses of studies on forest sprout regeneration. *Scientia Silvae Sinicae* 43, 74–82, doi:10.3321/j.issn:1001-7488.2007.09.015 (2007).
27. Pyttel, P. L., Fischer, U. F., Suchomel, C., Gärtner, S. M. & Bauhus, J. The effect of harvesting on stump mortality and re-sprouting in aged oak coppice forests. *Forest Ecology and Management* 289, 18–27, doi:10.1016/j.foreco.2012.09.046 (2013).
28. Berrill, J.-P., Schneider, K., Dagley, C. M. & Webb, L. A. Understory light predicts stump sprout growth in mixed multiaged stands in north coastal California. *New Forests* 49, 815–828, doi:10.1007/s11056-018-9636-6 (2018).
29. Bartušková, A., Filartiga, A. L., Herben, T., Qian, J. & Klimešová, J. Comparative analysis of root sprouting and its vigour in temperate herbs: anatomical correlates and environmental predictors. *Annals of Botany* 127, 931–941, doi:10.1093/aob/mcab030 (2021).
30. Yu, F.-H., Wang, N., He, W.-M., Chu, Y. & Dong, M. Adaptation of rhizome connections in drylands: increasing tolerance of clones to wind erosion. *Annals of botany* 102, 571–577, doi:10.1093/aob/mcn119 (2008).
31. De Witte, L. C. & Stöcklin, J. Longevity of clonal plants: why it matters and how to measure it. *Annals of Botany* 106, 859–870, doi:10.1093/aob/mcq191 (2010).
32. Klimešová, J., Herben, T. & Martínková, J. Disturbance is an important factor in the evolution and distribution of root-sprouting species. *Evolutionary Ecology* 31, 387–399, doi:10.1007/s10682-016-9881-0 (2016).
33. Beatty, G. E. & Provan, J. High clonal diversity in threatened peripheral populations of the yellow bird's nest (*Hypopitys monotropa*; syn. *Monotropa hypopitys*). *Annals of Botany* 107, 663–670, doi:10.1093/aob/mcr003 (2011).
34. Wang, A., Zhang, Q., Wan, D., YanzhuoYang & Liu, J. Nine microsatellite DNA primers for *Hippophae rhamnoides* ssp. *sinensis* (Elaeagnaceae). *Conservation Genetics* 9, 969–971, doi:10.1007/s10592-007-9416-x (2007).
35. Roiloa, S. R., Retuerto, R., Campoy, J. G., Novoa, A. & Barreiro, R. Division of labor brings greater benefits to clones of *Carpobrotus edulis* in the non-native range: Evidence for rapid adaptive evolution. *Frontiers in Plant Science* 7, doi:10.3389/fpls.2016.00349 (2016).
36. Zakaria, Z., Mohamed, A. R., Salleh, N. H. M. & Mansor, S. N. A. Total nitrogen content from earthworm (*Eisenia Foetida*) using the kjeldahl method. *IJUM Engineering Journal* 14, doi:10.31436/ijumej.v14i1.315 (2013).
37. Liu, C. *et al.* Soil bacterial communities of three types of plants from ecological restoration areas and plant-growth promotional benefits of *Microbacterium invictum* (strain X-18). *Frontiers in Microbiology* 13, doi:10.3389/FMICB.2022.926037 (2022).
38. Sareen, A., Gupta, R. C., Bansal, G. & Singh, V. Comparison of key mineral elements in wild edible fruits of *Ziziphus Mauritiana* and *Z. nummularia* using atomic absorption spectrophotometer (AAS) and flame photometer. *International Journal of Fruit Science* 20, S987-S994, doi:10.1080/15538362.2020.1774468 (2020).

39. Meng, P., Liu, J. & Bai, X. Selection of tree species by principal component analysis for abandoned farmland in southeastern Horqin Sandy Land, China. *Journal of Forestry Research* 33, 475–486, doi:10.1007/s11676-021-01320-0 (2021).
40. Müllerová, J., Hédli, R. & Szabó, P. Coppice abandonment and its implications for species diversity in forest vegetation. *Forest Ecology and Management* 343, 88–100, doi:10.1016/j.foreco.2015.02.003 (2015).
41. Buckley, P. Coppice restoration and conservation: a European perspective. *Journal of Forest Research* 25, 125–133, doi:10.1080/13416979.2020.1763554 (2020).
42. da Silveira Pontes, L. *et al.* The role of plant traits and their plasticity in the response of pasture grasses to nutrients and cutting frequency. *Annals of botany* 105, 957–965, doi:10.1093/aob/mcq066 (2010).
43. Nakahara, T., Fukano, Y. & Yahara, T. Effects of apical damage on plant growth and male and female reproductive investments in *Ambrosia artemisiifolia*, a wind-pollinated plant. *Plant Ecology* 219, 853–862, doi:10.1007/s11258-018-0839-x (2018).
44. Kawamoto, H., Saito, T. & Kiritani, K. A soybean growth model based on compensatory growth following defoliation. *Ecological Research* 1, 195–206, doi:10.1007/BF02347021 (1986).
45. Oosterheld, M. & McNaughton, S. Effect of stress and time for recovery on the amount of compensatory growth after grazing. *Oecologia* 85, 305–313, doi:10.1007/BF00320604 (1991).
46. Bai, S. *et al.* Response of stump sprouting ability to endogenous hormones in *Hippophae rhamnoides* ssp. *sinensis*. *Journal of Southwest Forestry University* 40, 82–87, doi:10.11929/j.swfu.201912043 (2020).
47. Zhang, Z. *et al.* Endogenous phytohormone response to stumping treatments in *Hippophae rhamnoides* ssp. *sinensis* *Journal of West China Forestry Science* 45, 29–36, doi:10.16473/j.cnki.xblykx1972.2019.05.005 (2019).
48. Maschinski, J. & Whitham, T. G. The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. *The American Naturalist* 134, 1–19, doi:10.2307/2462272 (1989).
49. Yang, Z., Minggagud, H., Baoyin, T. & Li, F. Y. Plant production decreases whereas nutrients concentration increases in response to the decrease of mowing stubble height. *Journal of Environmental Management* 253, 109745, doi:10.1016/j.jenvman.2019.109745 (2020).
50. Zhang, Z. *et al.* Response of root suckering ability of *Hippophae rhamnoides* ssp. *sinensis* to stubble height. *Journal of Northeast Forestry University* 49, 21–25, doi:10.13759/j.cnki.dlxb.2021.03.004 (2021).
51. Tian, D. *et al.* Response of root suckering ability and non-structural carbohydrates accumulation of Chinese sea buckthorn to stubble height. *Journal of Northwest A&F University* 51, 1–14, doi:10.13207/j.cnki.jnwafu.2023.09.008. (2023).
52. Gleason, S. & Ares, A. Photosynthesis, carbohydrate storage and survival of a native and an introduced tree species in relation to light and defoliation. *Tree Physiology* 24, 1087–1097,

doi:10.1093/treephys/24.10.1087 (2004).

53. Asao, S. & Ryan, M. G. Carbohydrate regulation of photosynthesis and respiration from branch girdling in four species of wet tropical rain forest trees. *Tree Physiology* 35, 608–620, doi:10.1093/treephys/tpv025 (2015).
54. Li, G., Tang, D. & Zhao, Y. The biological property and ecological habit of *Hippophae*. *Acta Botanica Boreal-Occidentalia Sinica* 20, 892–897, doi:10.3321/j.issn:1000-4025.2000.05.034 (2000).
55. Canadell, J. & López-Soria, L. Lignotuber reserves support regrowth following clipping of two Mediterranean shrubs. *Functional Ecology* 12, 31–38, doi:10.1046/j.1365-2435.1998.00154.x (1998).
56. PATE, J. S., FROEND, R. H., BOWEN, B. J., HANSEN, A. & KUO, J. Seedling growth and storage characteristics of seeder and resprouter species of Mediterranean-type ecosystems of SW Australia. *Annals of Botany* 65, 585–601, doi:10.2307/2444374 (1990).
57. Vesk, P. A., Warton, D. I. & Westoby, M. Sprouting by semi-arid plants: testing a dichotomy and predictive traits. *Oikos* 107, 72–89, doi:10.1111/j.0030-1299.2004.13122.x (2004).
58. Kays, J. S. & Canham, C. D. Effects of time and frequency of cutting on hardwood root reserves and sprout growth. *Forest Science* 37, 524–539, doi:10.1093/forestscience/37.2.524 (1991).

Figures

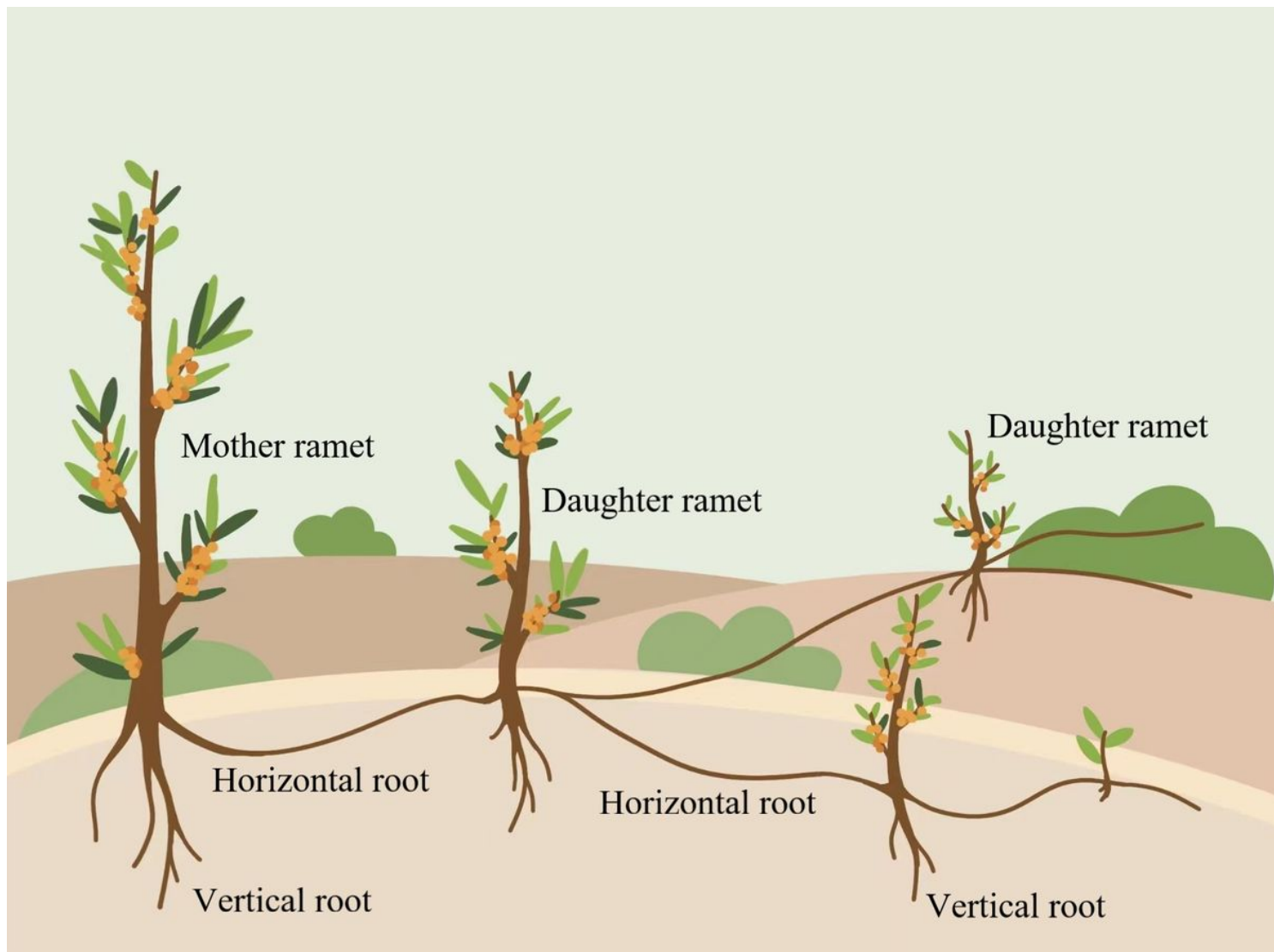


Figure 1

Schematic diagram of root sprouting and clone dispersal of *H. r. ssp. sinensis*.

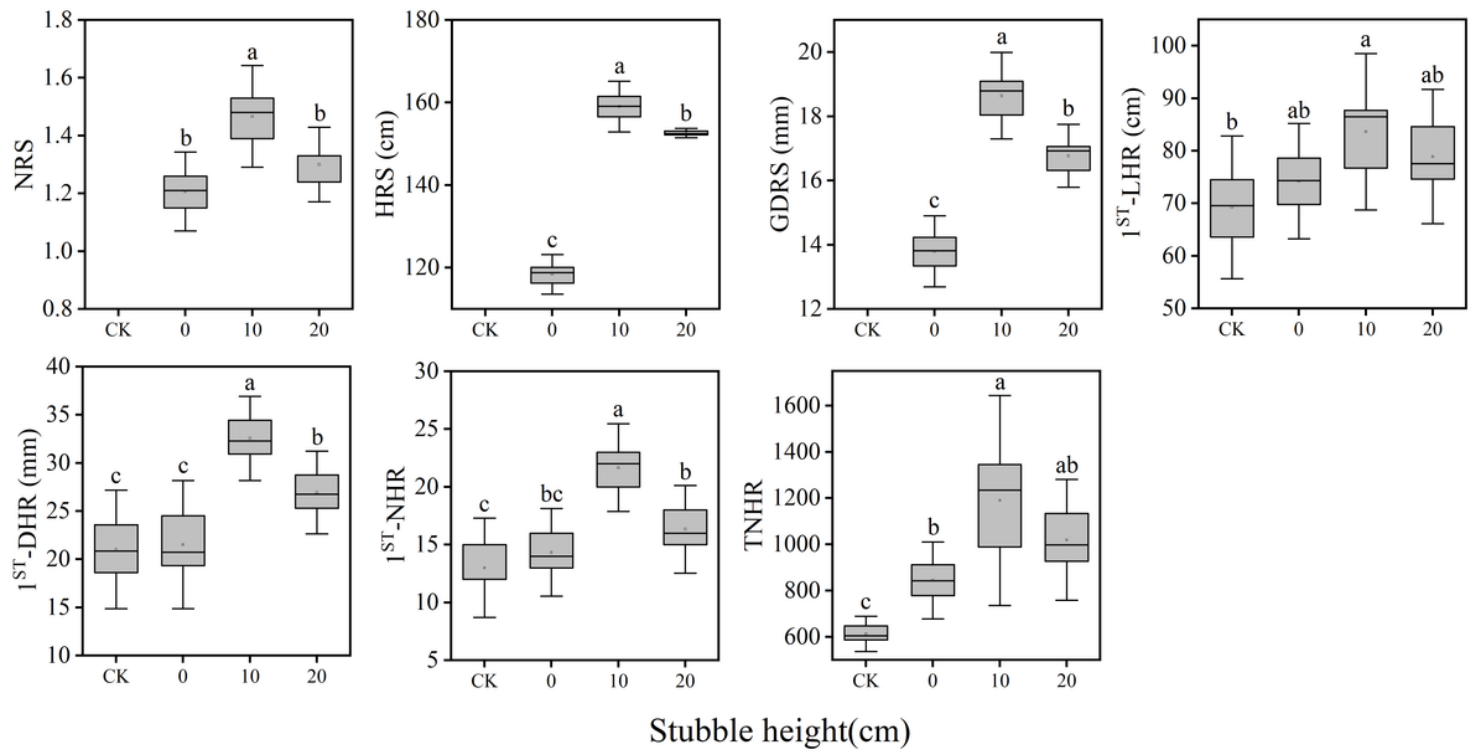


Figure 2

Significance analysis of differences in root sprouting and clonal dispersal ability at different stubble heights. NRS, number of root sprouts; HRS, height of root sprouts; GDRS, ground diameter of root sprouts; 1st-LHR, length of the first-order horizontal root; 1st-DHR, diameter of the first-order horizontal root; 1st-NHR, number of first-order horizontal roots; TNHR, total number of horizontal roots. Different lowercase letters indicate significant difference ($P < 0.05$), same below. Bars represent standard deviation (SD).

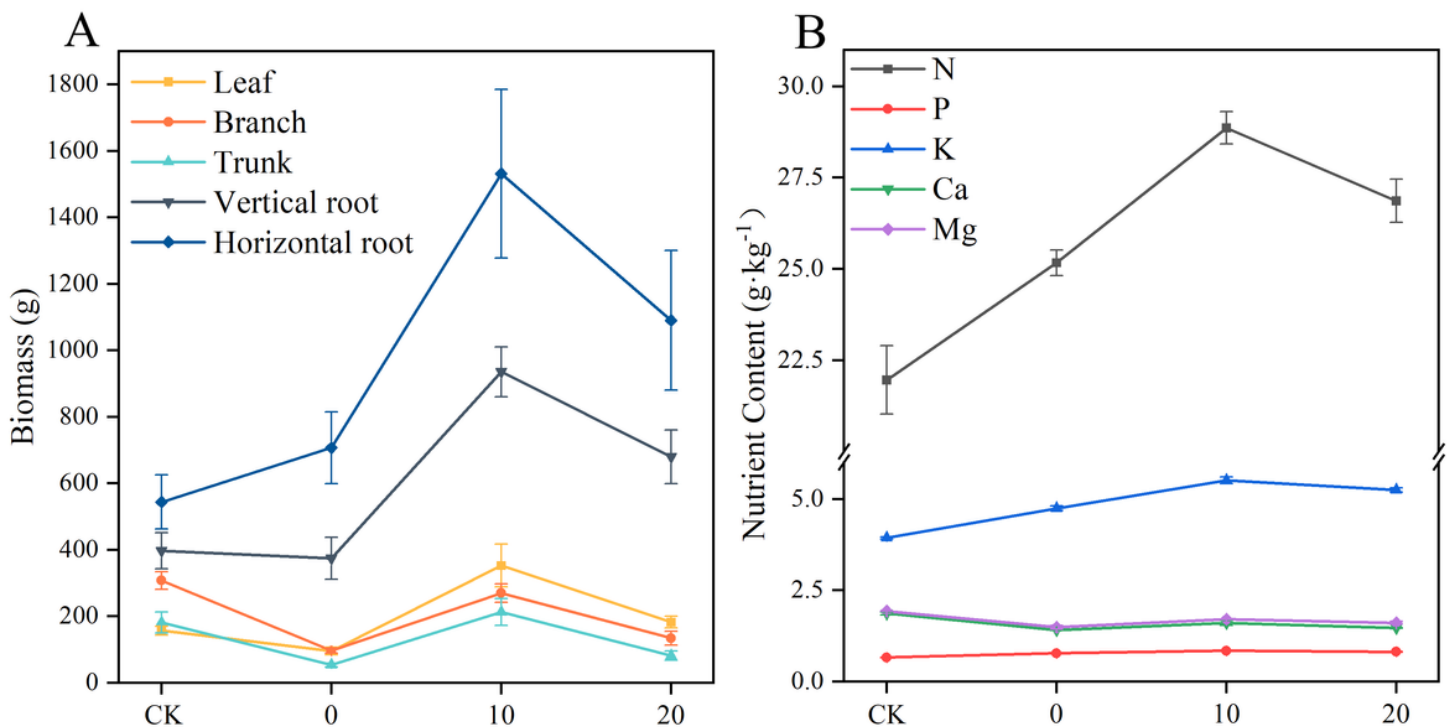


Figure 3

Significance analysis of differences in biomass (A) and nutrient content (B) at different stubble heights. Bars represent SD.

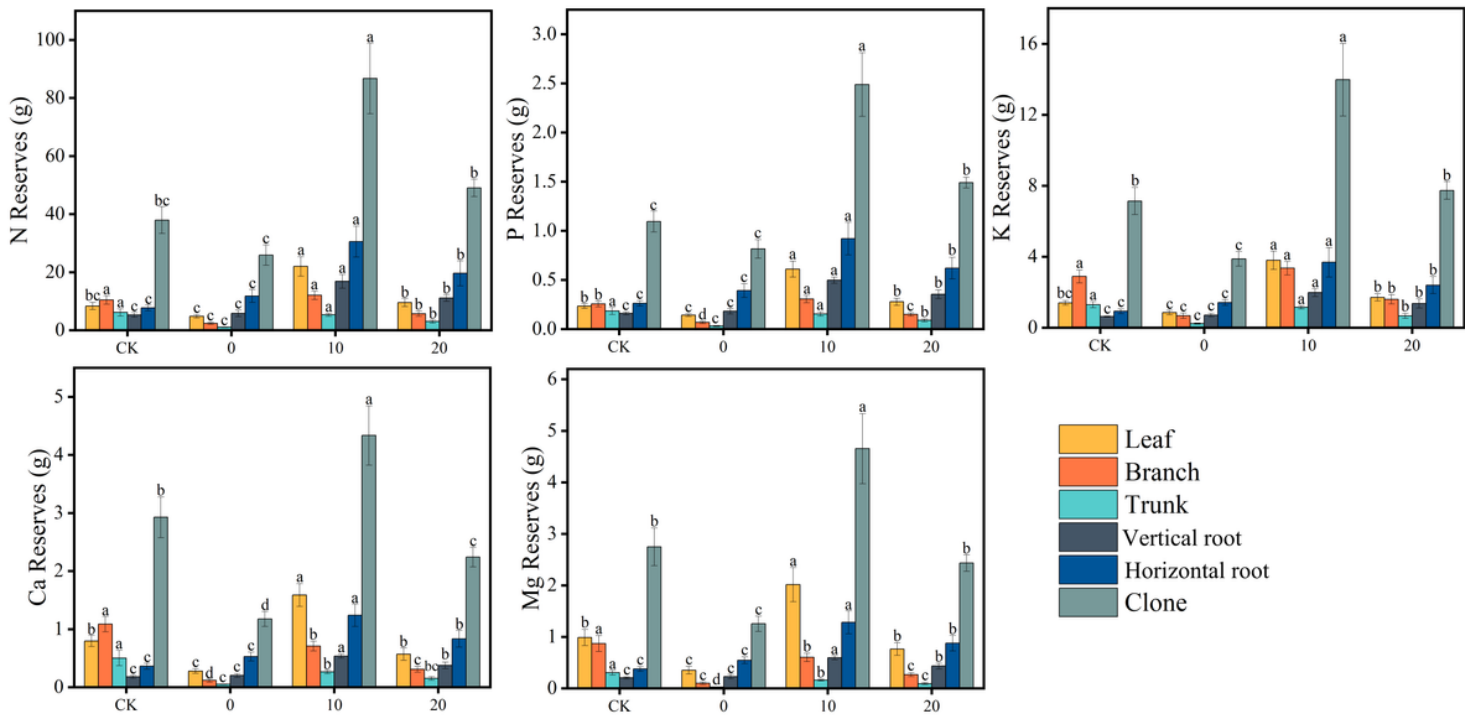


Figure 4

Cloning of different stubble height and nutrient element accumulation in each component. Bars represent SD.

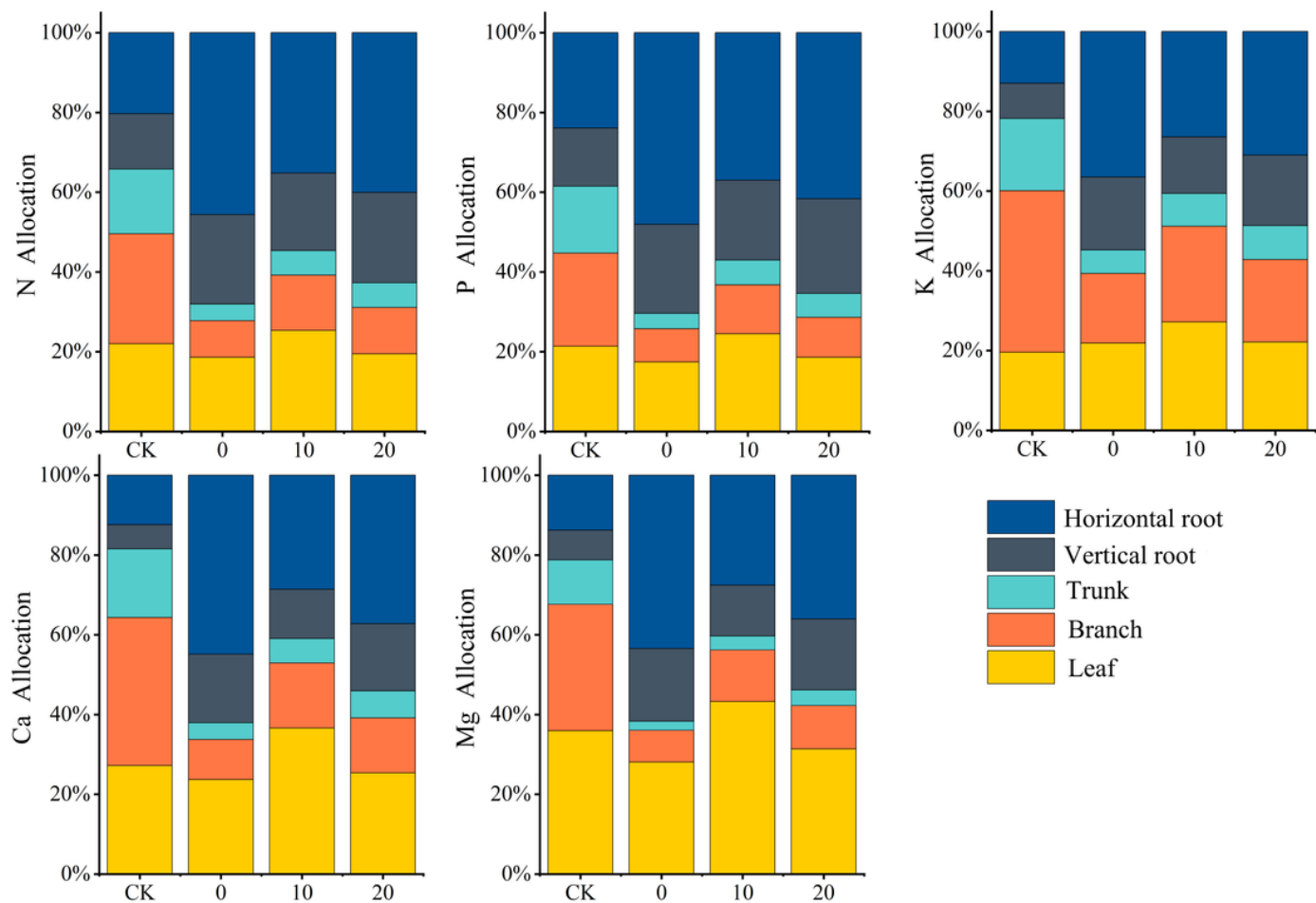


Figure 5

Effect of different stubble height on the allocation of nutrients to the clone and each component.

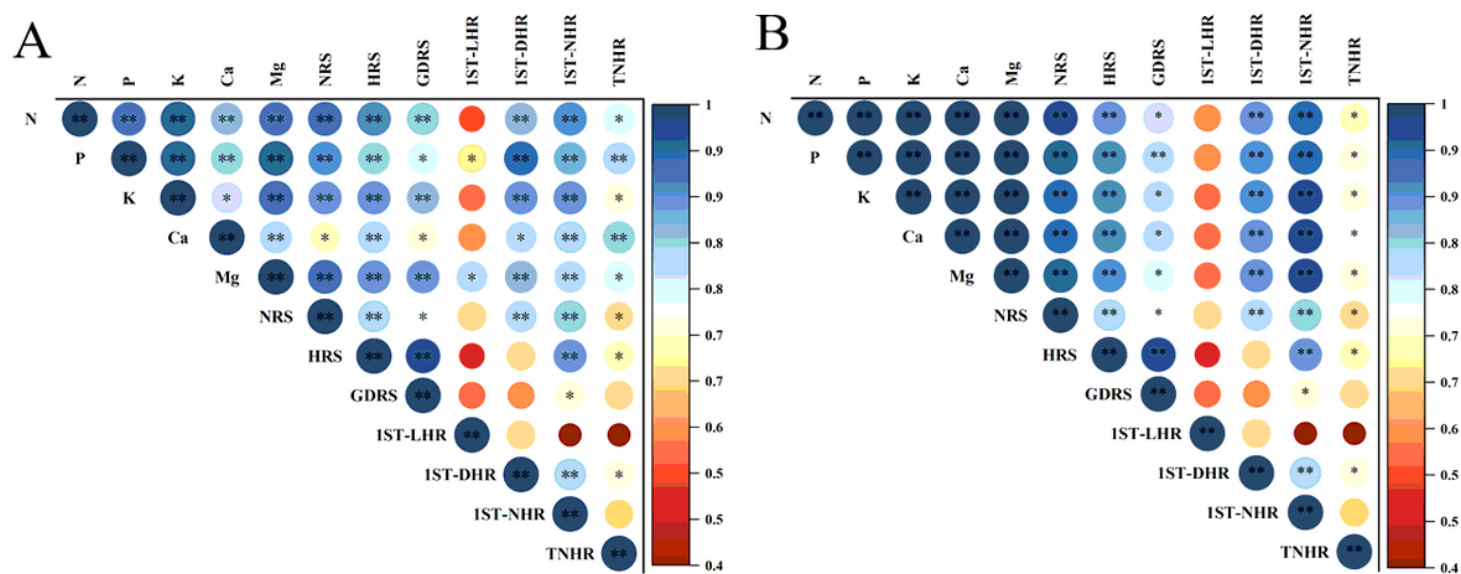


Figure 6

Correlation analysis of root sprouting plant and clonal dispersal capacity with nutrient content (A) and reserves (B). *, $P \leq 0.05$; **, $P \leq 0.01$. Phenotypic trait abbreviations are shown in Figure 3.

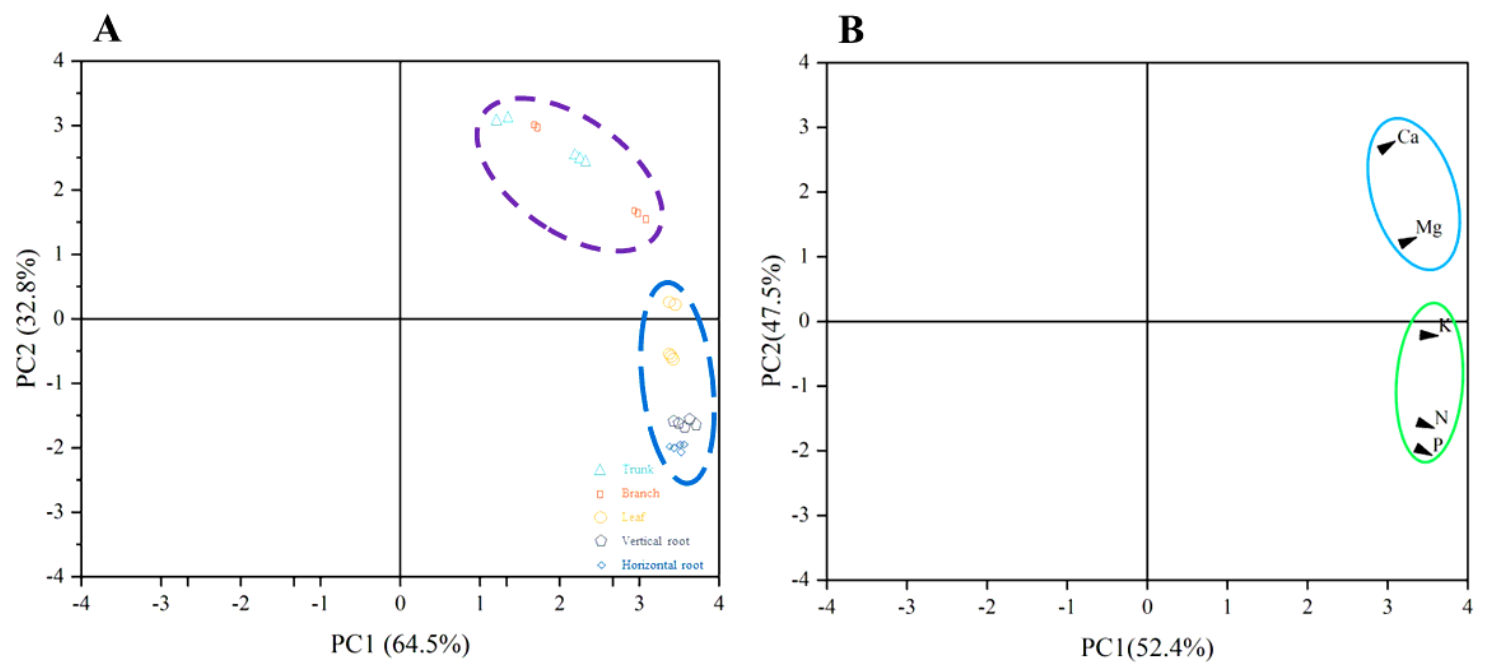


Figure 7

Biplots of the first two components of different clone components (A) and different nutrients (B).

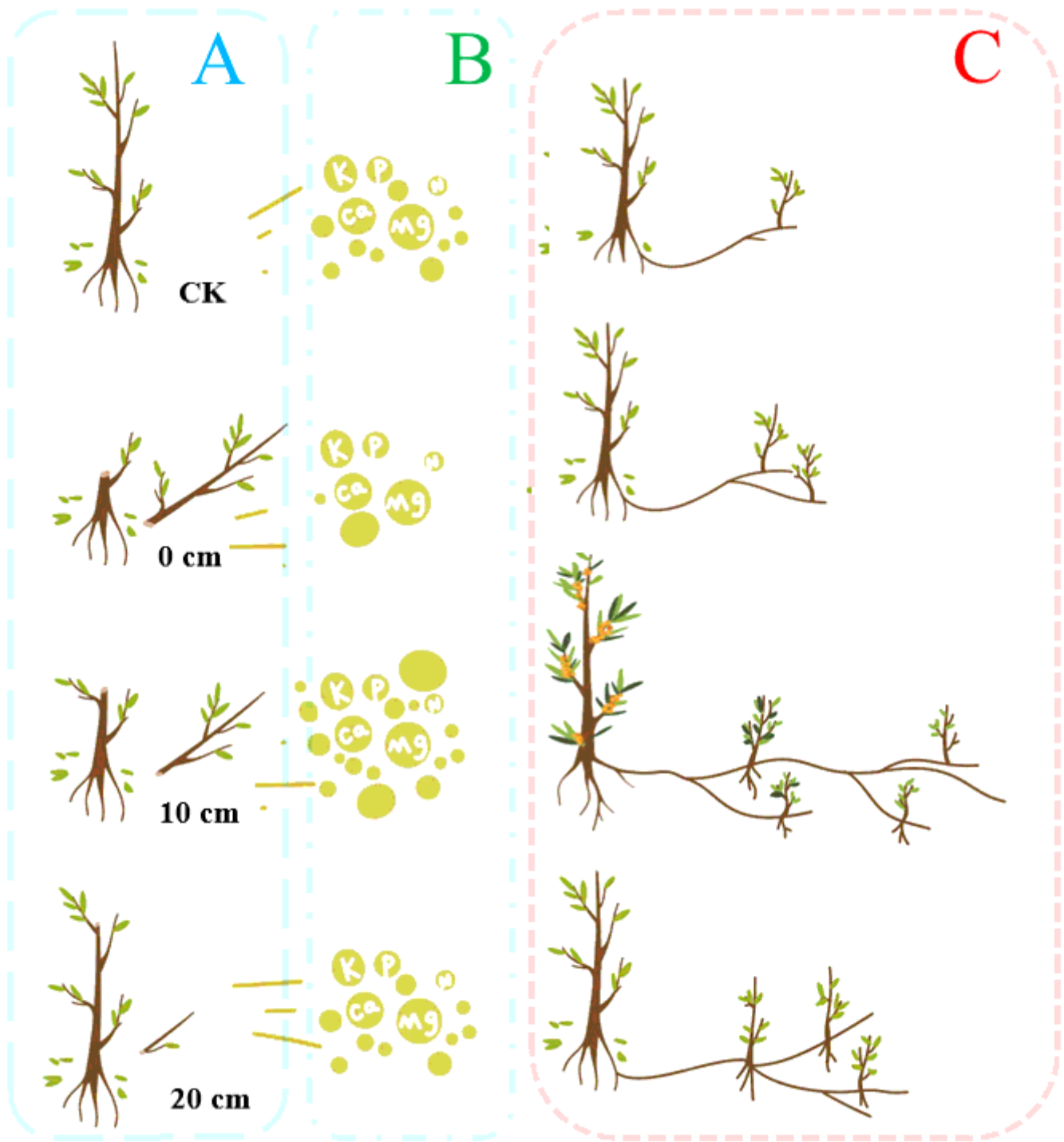


Figure 8

Schematic diagram for effect of stubble height on root sprouting and clonal dispersal ability of *H. r. ssp. sinensis* and its nutrient accumulation and allocation. A: Coppicing at different heights. B: Nutrient accumulation and allocation. C: Root sprouting and clonal dispersal. The number of bubbles indicates the accumulation of nutrient.