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YongQin CUI
1 Dunhuang Gobi and Desert Ecology and Environment Research Station, Cold and Arid Regions Environmental and Engineering Research Institute, CAS, Lanzhou 730000, China;

JianYing MA
1 Dunhuang Gobi and Desert Ecology and Environment Research Station, Cold and Arid Regions Environmental and Engineering Research Institute, CAS, Lanzhou 730000, China;, jyma@lzb.ac.cn

Wei SUN
2 Key Laboratory of Vegetation Ecology, Ministry of Education, Institute of Grassland Sciences, Northeast Normal University, Changchun 130024, China

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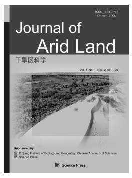

Application of stable isotope techniques to the study of soil salinization

YongQin CUI[1], JianYing MA[1*], Wei SUN[2]

[1] Dunhuang Gobi and Desert Ecology and Environment Research Station, Cold and Arid Regions Environmental and Engineering Research Institute, CAS, Lanzhou 730000, China;

[2] Key Laboratory of Vegetation Ecology, Ministry of Education, Institute of Grassland Sciences, Northeast Normal University, Changchun 130024, China

Abstract: In this paper, we reviewed the progress in the application of stable isotope techniques to the study of soil salinization. As a powerful technique, stable isotopes have been widely used in the studies of soil water evaporation, the dynamics of soil salinization and salt-tolerant plant breeding. The impact of single environmental factors on plant isotope composition has been the focus of previous studies. However, the impact of multiple environmental factors on plant isotope composition remains unclear and needs to be carefully studied. In order to gain insights into soil salinization and amelioration, especially soil salinization in arid and semiarid areas, it is essential to employ stable isotope techniques and combine them with other methods, such as located field observation and remote sensing technology.

Keywords: stable isotope; soil water evaporation; soil salinization dynamic; salt-tolerant plants breeding

Soil salinization is the accumulation of water-soluble salts in the topsoil, or regolith, which impacts agricultural production, environmental health, and economic welfare. Soil salinization is a serious environmental problem in many arid and semi-arid regions of the world (Huang, 2000; Dong *et al.*, 2005). Salinization is a common phenomenon of land degradation, influenced by such factors as climate conditions, soil parent material, water table, soil salinity, etc. In recent years, with a growing world population, there has been an increase in magnitude and intensity of salt-affected soils due to unreasonable irrigation methods, overuse of chemical fertilizer, severe deforestation, etc. It is estimated that tens of millions of hectares of land are degraded every year (Sanders, 2000). Soil salinization has attracted the attention of the government and scientists of many countries. At present, field observation has been widely used to study the physical and chemical properties of saline soil (Li *et al.*, 2004; Feng *et al.*, 2007; Lin and Dilbar, 2007; Zhang and Feng, 2009), the transport features of soil water and salt (Jiang *et al.*, 2006; Yu and Rui, 2007), the driving mechanisms of salinization (Zhang, 1993; Li, 2000; Tian, 2000; Fan *et al.*, 2001; Zhang and Wang, 2002; Ren and Xu, 2003; Chen *et al.*, 2007; Mamat *et al.*, 2008; Zhao *et al.*, 2008; Guan *et al.*, 2009; Wang *et al.*, 2009) and soil amelioration (Yu and Chen, 1994; Zhang and Wang, 1997; Li *et al.*, 1999; Li *et al.*, 2005; Liu *et al.*, 2005; Li *et al.*, 2006; Yu *et al.*, 2009)

As one of the most powerful tools used in ecological and environmental sciences, many stable isotope methods have been used to monitor the ecological process at different spatial and temporal scales, or address issues that are intractable using other methods. Currently, stable isotope methods are widely used in plant physiology (Metzner, *et al.*, 2010), zoology (Graves and Romanek, 2009) and microbial ecology (Bombach *et al.*, 2009) to do scientific research ranging from the molecular level to the entire ecosystem (Lin, 2010). In this paper, we briefly review the pro-

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*Corresponding author: JianYing MA (E-mail: jyma@lzb.ac.cn)

gress of the application of stable isotope techniques to the study of soil salinization, including the causes of soil salinization, soil salinization dynamic (Yang *et al.*, 2006) and salt-tolerant plants breeding (Poss *et al.*, 2000). Finally, we make some suggestions for the use of stable isotope techniques to study saline soil.

1 Stable isotopes: an introduction

Stable isotopes are nonradioactive atoms whose nuclei contain the same number of protons but a different number of neutrons. The major elements used in environmental studies include carbon, hydrogen, oxygen, nitrogen and sulfur. Isotopes behave similarly to these elements. However, certain differences exist in physico-chemical properties due to mass differences. These differences can lead to considerable separation of isotopes during the physical process and chemical reaction (Lin and Ke, 1995). Isotope effects are categorized primarily in two types; equilibrium fractionation and kinetic fractionation. Equilibrium isotope fractionation occurs during isotope exchange reactions that convert one phase (e.g., liquid) to another phase (e.g., vapor). Kinetic isotope fractionation occurs when the reaction is unidirectional and the reaction rates are sensitive to atomic mass at a particular position in one of the reacting species. Isotope effects are usually expressed in terms of delta (δ) values or discrimination values (Δ). Isotopic composition (δ) of a sample is an isotope ratio compared to a standard,

$$\delta X\,(‰) = (R_{sam}/R_{std} - 1). \quad (1)$$

Where, δX is the isotope ratio in delta units relative to a standard, and R_{sam} and R_{std} are the absolute isotope ratios of the sample and standard, respectively. Discrimination value (Δ) is the deviation of α from unity,

$$\Delta = \alpha - 1. \quad (2)$$

Where, α is a fractional factor (Mckinney *et al.*, 1950).

2 Application of stable isotope techniques to the study of soil salinization

2.1 Stable hydrogen and oxygen isotopes and evapotranspiration

Low rainfall and high evaporation lead to soil salinization in arid and semi-arid areas, and high evaporation affects the soil water-salt transport process directly (Qi *et al.*, 1997; Zhang *et al.*, 2008). High evaporation brings the salt to the ground surface with the aid of a capillary force, and then the salt stays in the ground surface and the shallow earth due to low leaching.

The application of hydrogen and oxygen stable isotopes to the study of soil moisture evaporation rule dates back to the 1960s. Zimmermann *et al.* (1967) first described deuterium isotope concentration in the saturated uniform sandy soil water on the condition that the soil evaporation in steady state has a constant temperature. The results show that the maximum deuterium isotope concentration occurs near the ground surface and decreases exponentially with increasing soil depth. Moreover, Barnes and Allison (1983) studied the motion model of the hydrogen and oxygen stable isotopes in evaporation of unsaturated soil under steady-state conditions and constant temperature, and found that the hydrogen and oxygen stable isotopes concentration increased with the soil depth until the evaporation front and then decreased exponentially, being similar to the above hydrogen and oxygen stable isotopic profile line, and that isotopic depletion might appear below the evaporation front under steady-state conditions and inconstant temperature (Barnes and Allison, 1984). Moreover, in the field, the soil evaporation is not steady-state in most cases. By the late 1980s, Walker *et al.* (1988) demonstrated that the motion of hydrogen and oxygen stable isotopes in unsaturated soil under unsteady-state conditions follow the energy conservation equation and mass conservation equation, and divided the soil evaporation into two stages: (1) soil stable isotopic profile began to shape when the evaporation rate was constant; (2) topsoil became dry when evaporation ratio decreased, and was in proportion to the square root of time; the accumulation evaporation increased, and was in inverse proportion to the square root of time.

A large number of researches of hydrogen and oxygen stable isotopes were conducted by domestic scholars. For example, Hu *et al.* (2008) considered surface evaporation, the most important reason for isotope enrichment in soil water after modeling the isotopic profile spread in saturated or unsaturated soil with the evaporation-fractionation model of soil isotope. Wang *et al.* (2010) presented evaporation as the main cause, which led to the fractionation of stable isotopes in soil water based on both Fick's law and mass conservation law.

Overall, the evaporation ratio of soil water in relation to the hydrogen and oxygen isotope discrimination, i.e. the hydrogen and oxygen isotope abundance increases with the increase of the evaporation rate. In addition, varing salinization degrees and types have different effects on the moisture dynamics in topsoil, which allows us to investigate the moisture dynamic mechanism and its effect on the salinization formation.

2.2 Stable isotopes and soil salinization

The change of $\delta^{13}C$ in soil carbonates or plants may reflect the soil salinization degree (Robert *et al.*, 1980; Yang *et al.*, 2006). Farquhar *et al.* (1989) reported that $\delta^{13}C$ increased with rising salinization degree whether the plant is halophyte or not, which was also proven by Yang *et al.* (2006). So the quantified categorization of salinization soil might proceed with the researches.

Salinization is a dynamic process (Yang *et al.*, 2006). The decrease of vegetation cover resulting from unreasonable human activities, i.e. over-clearing land, overgrazing, and denudation, aggravates evaporation in the topsoil, resulting in salt accumulation in the ground surface. During the dynamic process, the decrease of carbon dioxide with ^{12}C, and the increase of salts leads to carbon isotope fractionation of soil carbonate, resulting in the increase in soil $\delta^{13}C$ (Fig. 1). Hence, $\delta^{13}C$ values of soil carbonate reflects the changeable process and trend, with a positive relationship between the degree of salinization and $\delta^{13}C$ values of soil carbonate. Finally, by using $\delta^{13}C$ value in soil carbonate as an indicator to study salinization, we can understand a more objective and precise dynamic process of salinization.

2.3 Stable isotopes and breeding salt-tolerant plants

Plant growth in salt-affected land is frequently limited by high salt content, which is one of the major abiotic factors affecting crop yield in arid and semiarid areas (Shaheen and Hood-Nowotny, 2005). With the reduction of arable land, scientists have used various methods to ameliorate the saline soil, including effective land and water resource management practices and chemical ameliorations. However, these measures are expensive and the salinization recurs often. Biological ameliorations, especially salt-tolerant plant breeding, have become a research hotspot in recent years (Li *et al.*, 2003; Zhao and Fan, 2005; Lv *et al.*, 2010), which is of great importance to soil amelioration. Stable isotope methods have also been applied to screening salt-tolerant plants.

2.3.1 $\delta^{13}C$ and salt stress

The effect of salt stress on plants is mainly in the inhibition of plant growth by restricting photosynthesis. Stomatal closure is typically associated with increased salinity. Thus, the photosynthesis rate decreases while the salinity is higher than the optimum level (Sibole *et al.*, 1998; Wang *et al.*, 2009). There is a negative correlation between $\delta^{13}C$ value of plant and CO_2 mole fraction in the intercellular air space for plants growing under different saline envrionments (Wei *et al.*, 2008). Therefore, $\delta^{13}C$ value of plant can be used to reflect the magnitude of salt stress.

Foliar $\delta^{13}C$ values of C_3 plants have also been used as an integrated measure of the response of photosynthetic gas exchange to environmental variables such as salinity (Guy *et al.*, 1986). Farquhar *et al.* (1982) observed that C_3 halophytes growing at high salinity and low water potential had less negative $\delta^{13}C$ values, which was due to the decreasing of C_i/C_a, the ratio of intercellular and ambient CO_2 partial pressures. Qian *et al.* (2004) evaluated leaf carbon isotope discrimination as affected by salinity among three Kentucky bluegrass (*Poa pratensis* L.) cultivars that differ in their salt tolerance, and concluded that salinity induced a greater degree of stomatal resistance that provided less opportunity for discrimination against the heavier isotope. Shaheen and Hood-Nowotny (2005a; 2005b) achieved similar results through measuring $\delta^{13}C$ values of rice and wheat cultivars in different levels of salinity. In summary, salinity leads to a reduction in photosynthetic carbon isotope discrimination (Δ), and subsequently, an increase in $\delta^{13}C$ values. However, opposite conclusions were also reported by Chen *et al.*, (2004). Clough and Sim (1989) suggested that with increasing salinity, maximum photosynthesis and water use efficiency (WUE) of some mangrove plant species decreased, which are positively correlated with $\delta^{13}C$ values. The relationship between $\delta^{13}C$ values and salinity is relevant to intrinsic salt-tolerance,

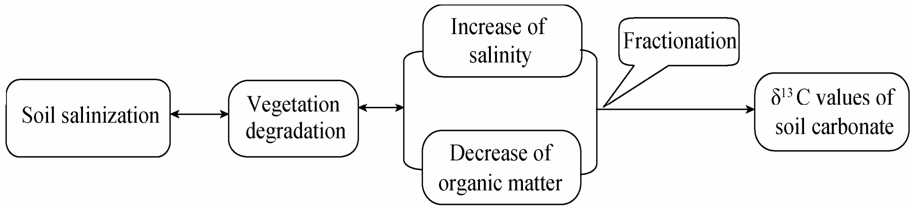

Fig. 1 Relationship between the carbon isotope compositions ($\delta^{13}C$) of soil carbonates and soil salinization

salinity level and the growth period of plant species under saline conditions (Wei *et al.*, 2008).

Relatively little information is available concerning the effects of salinity on photosynthetic gas exchange and carbon isotope compositon in C_4 herbs (Meizer *et al.*, 1994). Previous research has found that increasing soil salinity resulted in a decreased photosynthesis in the C_4 plants. A concomitant increase in O_2 sensitivity to photosynthesis and a decrease in $\delta^{13}C$ of leaves were also measured with increasing salinity. The results suggest that an increase in the leakage of CO_2 out of, and O_2 into the bundle sheath under saline conditions, led to an increase in the proportion of ^{13}C discriminated by RuBP carboxylase (Bowman *et al.*, 1989). By measuring carbon isotope discrimination, and gas exchange, Meinzer *et al.* (1994) suggested that variation in $\delta^{13}C$ value was attributed largely to the variation in bundle sheath leakiness to CO_2 (φ). Salinity-induced increases in φ appeared to be caused by a reduction in the C_3 pathway activity relative to C_4 pathway activity (Meinzer *et al.*, 1994). According to former research, there is a negative correlation between the $\delta^{13}C$ value of the C_4 plant and salinity. Further research is clearly needed on both the mechanisms of stress-induced adjustments in photosynthesis and on the extent of which the $\delta^{13}C$ values in C_4 plant is influenced by the increase in the leakage of CO_2.

2.3.2 $\delta^{13}C$ and breeding salt-tolerant plants

There are two main mechanisms of plant salinity tolerance. One mechanism is to prevent salt injury through salt exclusion, salt rejection and salt dilution; another is to endure the saline environment by changes in plant characteristics, including osmotic stress-tolerance, balance of elements and stability of the metabolism (Cheeseman, 1988; Yang *et al.*, 2002). So breeding salt-tolerant plants can improve the salt-tolerant plants, taking full advantage of the saline soil (Wang, 2007) and increasing the crop yield. Stable isotope techniques can be applied to the selection of a salt-tolerant species.

As mentioned above, foliar $\delta^{13}C$ values have been used as an integrated measure in the response of photosynthetic gas exchange to environmental variables such as soil water availability, light, humidity and salinity. Thus, $\delta^{13}C$ value may serve as a useful selection criterion in breeding efforts to develop salt tolerant plants. When screening salinity tolerance in rice at the seedling stage, there was a significant negative relationship between the Δ value and the salt stress of rice, which indicated that a high Δ value could be used as a physiological indicator for screening the tolerant cultivars of rice (Shaheen and Hood-Nowotny, 2005a). The study of Meinzer *et al.* (1994) showed that the CO_2 assimilation rate (A), stomatal conductance (g_s) and shoot growth rate (SGR) of sugarcane began to decline as electrical conductivity (EC) of the irrigation solution increased above 2 dS/m. A, g_s, and SGR of a salt-resistant cultivar were consistently higher than those of a salt-susceptible cultivar at all levels of salinity and declined less with increasing salinity. The $\delta^{13}C$ value in tissues obtained from the uppermost fully expanded leaf decreased with salinity. Therefore, the $\delta^{13}C$ value may be used as an indicator for A, g_s and SGR and for screening salt-tolerant sugracane. Qian *et al.* (2004) reported that salinity induced a greater degree of stomatal resistance that provided less opportunity for discrimination against the heavier isotope, and carbon isotope discrimination is a useful criterion for screening salt-tolerant Kentucky bluegrass cultivars. It was also concluded that the $\delta^{13}C$ value may prove to be a useful index for selecting wheat cultivars with improved harvest index in salt-affected areas (Shaheen and Hood-Nowotny, 2005b).

3 Conclusion and suggestions

Stable isotope technology has started to show its great potential as a powerful tool in researching saline soil formation mechanism and breeding salt-tolerant plants.

The $\delta^{13}C$ value can reflect the developing tedency of soil salinization and indicate the effects of salt stress on plants, and can be used as a useful indicator for screening salt-tolerant plants. In the meantime, stable isotope technology has some defects during the application process. For example, the cost of sample analysis is expensive because the mass spectrometer used to obtain isotopic ratios, needs to be professionally operated. Currently, the application of stable isotope technology on the study of soil salinization has little field experimentation, and is still in the theorectically stage. Therefore, we have the following suggesitons for the use of isotopes to research saline soils.

(1) The application of stable isotopes to the study of soil salinization is a process from simple to complex and from monofactor to multifactor. One of its development trends is to analyze the effects of multiple environmental factors, such as temperature (O'leary, 1981), light (Yin *et al*., 2008), precipitation (Sun *et al*., 2003), atmospheric CO_2 concentration (Feng and Epstein, 1995), altitude (Chen *et al*., 2003), latitude and altitude (Ma *et al*., 2005), as well as regional background. The regionalization and comprehensiveness of the study on saline soil help to improve prediction, to mornitor the dynamics of soil salinizaton, and is an important basis of soil amelioration.

(2) It is beneficial to resolve the scientific issues if combining the isotopes with other methods, such as field investigaton or remote sensing (Jiang *et al*., 2008; Wang and Tashpolat, 2009). Combining stable isotope techniques with other branch fields of ecology is conducive to exploring the cycling of matter at the terrestrial ecosystem level and the process of vegetation degradation. For instance, soil salinization can be researched by using hydrogen and oxygen stable isotopes (Wang *et al*., 2008) while understanding the relationship between soil water and soil salinity (Li *et al*., 2000).

(3) It is necessary to seek the substitution indices of $\delta^{13}C$, because the system is quite expensive.

(4) The relationship between isotope discrimination and the stress-induced physiological changes in plants of growing under different degrees of salinity need to be established. Thus, sampling numerous plants under salinity stress conditions in different geographic units and climate conditions are necessary.

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