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# Different responses of non-structural carbohydrates in above-ground tissues/organs and root to extreme drought and re-watering in Chinese fir (*Cunninghamia lanceolata*) saplings

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## Abstract

Key message The total NSC concentration in the roots declined more significantly than in the above-ground tissues/organs under drought treatment, and the level did not return to that of the control after re-watering. Abstract Non-structural carbohydrates (NSC) reflect the relative balance between C-gain (photosynthesis) and C-loss (respiration) and play a pivotal role in carbon cycling in a forest ecosystem. However, little is known regarding the effects of extreme drought and re-watering on the NSC status in different tissues/organs. This study examined the variation in NSC concentrations in different tissues/organs and the total NSC pool sizes in Chinese fir (Cunninghamia lanceolata) saplings after drought and rewatering. Results showed that significant differences were observed in the concentrations of total NSC and its components in the different tissues/organs. For example, the NSC concentrations were nine times higher in bark than in stemwood. Moreover, the responses of NSC and its

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components to extreme drought also varied in different tissues/organs. Drought either significantly increased or maintained the total NSC concentration in the aboveground tissues/organs. By contrast, drought reduced the total NSC concentration in the sapling roots. Furthermore, the results also showed that extreme drought leads to sapling death, which is supported by the result of needle staining and the failure of the total NSC concentration to recover after re-watering. The concentrations of NSC and its components further decreased, and a more pronounced decline was observed in the roots than in the above-ground tissues/organs after re-watering. We speculated that drought can cause failure in carbon translocation between the above- and below-ground tissues/organs and thus cause varied responses of different tissues/organs to extreme drought and re-watering. Overall, these findings suggest the need to investigate the potential differential responses of various tissues/organs to climate change.

Keywords Non-structural carbohydrates (NSC)  $\cdot$  Drought  $\cdot$  Re-watering  $\cdot$  Chinese fir

# Introduction

Non-structural carbohydrates (NSC) play a pivotal role in carbon cycling in forest ecosystems; in particular, NSC acts as a buffer for insufficient source activity in trees (Hoch et al. 2003; Zhu et al. 2012; Yang et al. 2015), thereby allowing plant survival under stressful conditions (Poorter and Kitajima 2007; O'Brien et al. 2014) and facilitating recovery after disturbances (Iwasa and Kubo 1997). The concentration and pool size of NSC reflect the relative balance between C-gain (photosynthesis) and C-loss (respiration) (Chapin et al. 1990). Therefore, a comprehensive

understanding about the variation in NSC status and the response of NSC concentration to stressful conditions is critical in predicting the future carbon cycling as part of the effort to address global climate change (McDowell et al. 2008; Li et al. 2013).

The frequency and severity of drought events continue to increase because of climate change and unreasonable human activities (IPCC 2007). The response of carbon cycling in forest ecosystems to drought has recently received much attention (Zhao and Running 2010; Zeppel et al. 2011). During drought, trees downregulate the carbon assimilation rates by reducing stomatal conductance (Farquhar and Sharkey 1982, Zang et al. 2014), but drought does not significantly reduce maintenance respiration (Meir et al. 2008). Therefore, stored carbon reserves may be remobilized to fuel the maintenance respiration (McDowell et al. 2008; McDowell and Sevanto 2010). However, contradictory results on the responses of NSC to experimental drought have been observed. Some studies have reported that the NSC concentration increases during drought (Würth et al. 2005; Galvez et al. 2011; Liu et al. 2015), whereas other reports have found that the NSC concentration decreases (Sayer and Haywood 2006; Anderegg 2012; Adams et al. 2013) or remains constant (Pizarro and Bisigato 2010, Gruber et al. 2012) under the same condition.

These discrepancies can be partly attributed to the species-specific ability to reach different depths of water resources (Nardini et al. 2016), the severity of drought (Zhang et al. 2015) or the potential differential responses across different tissues/organs (e.g. leaves, bark, stemwood and roots) to drought (Hartmann et al. 2013a). Hartmann et al. (2013a) reported that in Norway spruce saplings, the effect of drought on the NSC concentration is less prominent in the above-ground tissues/organs than in the roots. Dichio et al. (2009) found that the NSC concentration in thin roots of olive saplings is more sensitive to drought than that in medium roots. However, the majority of recent studies focused on the effect of drought on the NSC status in individual tissues/organs, such as leaves (Rodríguez-Calcerrada et al. 2011; Adams et al. 2013; Liu et al. 2015) or roots (Galvez et al. 2011). To date, few studies have investigated the drought response of NSC concentration in different tissues/organs (Hartmann et al. 2013a), particularly to extreme drought and re-watering.

Chinese fir (*Cunninghamia lanceolata*) is a fast-growing timber species that has been widely planted to meet increasing timber demand. In recent decades, drought occurred frequently in subtropical China (Zhai et al. 2010; Lu et al. 2011; Yang et al. 2012), where Chinese fir plantations have been widely established. However, little is known about the effects of extreme drought on the NSC status in Chinese fir. Therefore, the present study examined

the variation in NSC concentrations among different tissues/organs and the total NSC pool size in Chinese fir saplings after exposure to extreme drought and re-watering treatments. This study aims to investigate the difference in NSC concentrations among different tissues/organs and to analyze the effects of drought and re-watering on the NSC concentrations in these tissues/organs. Our specific objectives are to test the following hypotheses: (1) there are significant differences in the NSC concentrations among different tissues/organs; and (2) the NSC concentration in roots is more sensitive to drought than above-ground tissues/organs.

# Materials and methods

### Study site and experimental design

This study was conducted in the Huitong Experimental Station of Forest Ecology (26°40'-27°09' N and 109°26'-110°08' E) of the Chinese Ecological Research Network in Huitong County, Hunan Province, China. The mean annual temperature of the site is 16.5 °C, with a monthly mean temperature ranging from 1.9 °C in January to 29.0 °C in July. The site receives an annual rainfall of approximately 1200 mm and 1 month to 2 months of summer drought that usually begins in late July (Wang et al. 2013). In late March 2013, 50 one-year-old Chinese fir saplings with local provenance were obtained from a nursery garden and transferred into plastic pots (diameter 35 cm; height 30 cm). These plastic pots were perforated at the bottom to allow water drainage. Plastic pots were filled with homogenized surface soil obtained from a Chinese fir plantation. A translucent roof with a height of 2.0 m was installed over the potted saplings to prevent throughfall. All pots were manually watered to field capacity for 2-3 days based on the amount of water lost via evapotranspiration, which is determined using the change in individual pot weight. All pots were randomly arranged daily to ensure the exposure of all saplings to homogeneous conditions.

In early July 2013, a total of 40 healthy saplings were selected in this drought and re-watering experiments. The selected sample saplings were relatively uniform based on sapling height and ground diameter. On July 20, 2013, eight randomly chosen saplings were harvested carefully, stored immediately in a cool box, and then transported into the laboratory for dry mass and NSC analyses. Afterward, drought was initiated. Two water regimens were imposed, namely, control and drought (suspension of watering). Up to 16 saplings per treatment were used for 30 days. On August 20, 2013, eight randomly chosen saplings in each treatment were harvested for laboratory dry mass and NSC analyses. The eight remaining saplings in the drought

treatment were watered and maintained at or near field capacity for re-watering treatment (drought-CK). The eight remaining saplings in the control treatment served as control (CK–CK). All saplings in the different treatments were harvested on September 10, 2013 for laboratory analysis. To minimize the diurnal variation in carbohydrate concentration (Morin et al. 2011), the saplings were harvested between 8:00 and 9:00 at each occasion. Throughout the drought and soil re-watering periods, volumetric water content was determined using a handheld time–domain reflectometer (TRIME-PICO TDR, Imko Company, Germany).

### **Biomass and NSC analyses**

In the laboratory, all saplings harvested on July 20, August 20, and September 10, 2013 were immediately separated into the needles, stems (including the axis of twigs) and roots. The needles were further categorized into current-year needles (CN) and 1-year-old needles (ON). Bark (BA) also was separated manually from stemwood (SW) for each stem sample. The roots were divided in two groups: coarse roots (with a diameter  $\geq 2$  mm; CR) and fine roots (with a diameter <2 mm; FR). All samples obtained from each tissue/organ were placed in the microwave at 600 W for 90 s to denature the enzymes (Hoch et al. 2003) and were subsequently oven heated at 70 °C for at least 72 h until a consistent weight was obtained. The biomass (dry mass) of each tissue was then recorded. Each dried sample was milled and stored at 4 °C prior to NSC analyses.

NSC analyses were conducted using four replicates for each treatment (4 randomly selected saplings from a total of 8 saplings). The NSC are defined here as soluble sugar (sucrose, glucose and fructose) plus starch. Soluble sugar and starch analysis was performed following the method described by Yang et al. (2015). About 0.1 g milled sample was suspended in 10 mL of aqueous ethanol (80 % v/v) and incubated in a water bath shaker at 80 °C for 10 min. The sample was centrifuged at 3000 rpm for 10 min. Supernatant and residues were obtained for soluble sugar and starch analyses. Soluble sugar was measured enzymatically by the K-SUFRG Kit (Megazyme, Wicklow, Ireland) (Bergmeyer et al. 1988; McCleary et al. 1997). Dglucose concentration was quantified using hexokinase and glucose-6-phosphate-dehydrogenase. Nicotinamide adenine dinucleotide phosphate (NADPH) absorbance was measured at 340 nm using a spectrophotometer (Hitachi Ltd, Tokyo, Japan). Subsequently, D-fructose concentration was determined following isomerization by phosphoglucose isomerase. Sucrose concentration is the difference in D-glucose content before and after sucrose hydrolysis by  $\beta$ fructosidase (invertase). If the amount of soluble sugars per sample is higher than the threshold concentration of NADPH, the sample solution must be diluted. Starch was measured enzymatically by the K-TSTA Kit (Megazyme, Wicklow, Ireland) (Bergmeyer et al. 1988; McCleary et al. 1997). Starch was hydrolyzed to maltodextrin by  $\alpha$ -amy-lase and was further hydrolyzed to D-glucose by amy-loglucosidase. Starch concentration was then determined by absorbance measurement at 510 nm after adding a glucose oxidase/peroxidase reagent.

#### Data analysis

One-way ANOVA was used to test the differences in the soluble sugar, starch, and total NSC concentrations among the different tissues/organs before drought treatment. Two-way ANOVA was used to test the effect of drought treatment, different tissues/organs, and their interaction on the total NSC and its components concentrations during the drought and rewatering periods. Whole-sapling NSC pool sizes (gram per sapling) were calculated as the sum of the NSC contents (the products of NSC concentrations and tissue biomasses.) in different tissues/organs. NSC fraction indicates the relative allocation of NSC content to different tissues/organs. All analyses were performed using SPSS software (SPSS 17.0 for Windows, SPSS Inc., Chicago, IL, USA).

## Results

## Soil water content

No significant difference in soil moisture was observed before drought treatment (P > 0.05). After 12 days of drought treatment, soil moisture decreased progressively with drought time, dropping by 51.8 and 56.2 % in drought and drought-CK treatments, respectively (Fig. 1). After 26 days of drought, the corresponding soil moisture was 80.8 and 81.6 % in drought and drought-CK treatments, respectively (Fig. 1). After re-watering, soil moisture returned to the control level (Fig. 1).

# Differences in NSC concentrations among tissues/ organs

Significant difference in soluble sugar concentration was observed among the tissues/organs (P < 0.05) harvested on July 20, 2013 (before drought treatment). The soluble sugar concentrations in different tissues/organs ranged from 13.91 mg/g to 135.74 mg/g and were found in the following increasing sequence: SW < FR < CR < CN < BA < ON (Fig. 2a). Significant difference in starch concentration was also observed among the tissues/organs (P < 0.05). However, the sequence of increasing starch concentration (SW < CN < ON < FR < BA < CR) was different from



Fig. 1 Variations in soil moisture in different treatments of Chinese fir saplings during drought (30 days) and subsequent re-watering (20 days). Values are mean  $\pm$  SE (n = 8). The beginning of drought treatment and re-watering is indicated by dashed line

that of soluble sugar concentration (Fig. 2b). A similar pattern of soluble sugar concentration was observed in total NSC concentration of the different tissues/organs because of the negligible contribution of starch to the total NSC. Similar to the soluble sugar concentration, we found that the total NSC concentration in BA was nearly nine times higher than that in SW (Fig. 2c).

# Response of NSC concentration to drought and rewatering

A significant interactive effect of drought and tissues/organs on soluble sugar concentrations was observed (Fig. 3a; Table 1), thereby indicating that the responses of soluble sugars of the different tissues/organs to drought significantly varied. No statistical difference (P > 0.05) in soluble sugar concentration in CN and ON was observed between CK and drought treatment. However, the soluble sugar concentration increased significantly in BA and SW and decreased significantly in CR and FR after drought treatment (Fig. 3a). A significant interactive effect of drought and tissues/organs on starch concentrations was observed (Fig. 3b; Table 1). In contrast to the soluble sugar concentration, the starch concentration in CN and ON showed a significant and pronounced decline (Fig. 3b). Moreover, the responses of the total NSC concentration to drought significantly varied among the different tissues/organs (Fig. 3c). The reduction in the total NSC concentration was more remarkable in the roots than in the needles and barks (Fig. 3c).

After re-watering, the soluble sugar, starch, and total NSC concentrations in the drought-CK treatment did not recover compared with the control levels (Fig. 4). The soluble sugar concentrations in all tissues/organs under the



Fig. 2 Soluble sugar (a), starch (b) and total NSC concentrations (c) in different tissues/organs of Chinese fir saplings before drought treatment (July 20, 2013). Values are mean  $\pm$  SE (n = 4). Different lowercase letters indicate significant differences among tissues/ organs (P < 0.05). CN current-year needles, ON 1-year-old needles, BA barks, SW stemwoods, CR coarse roots, FR fine roots

drought-CK treatment remained significantly lower (P < 0.01) than those under the CK–CK treatment, except in SW (P > 0.05), as indicated by a significant interactive effect of re-watering and tissues/organs (Table 2). Moreover, the change in the starch concentration was generally less dynamic in needles than in other tissues/organs (Fig. 4b; P < 0.05). The total NSC concentrations of all the tissues/organs further decreased after re-watering compared with those under drought treatment. Furthermore, the decline in the total NSC concentration was more pronounced in the roots than in the above-ground tissues/organs (Fig. 4c).



Fig. 3 Responses of soluble sugar (a), starch (b), and total NSC concentrations (c) in different tissues/organs of Chinese fir saplings to drought (August 20, 2013). Values are mean  $\pm$  SE (n = 4). The *asterisks* indicate significant differences between the control and drought treatments in individual tissue. \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001. *CN* current-year needles, *ON* one-year-old needles, *BA* barks, *SW* stemwoods, *CR* coarse roots, *FR* fine roots

#### **Total NSC pool and NSC fraction**

Drought and re-watering showed a negligible effect on biomass (Fig. 5a). Compared with the control treatment, the total NSC pool size in the drought treatment obviously decreased by 24.5 %. However, no significant difference was found in the total NSC pool size between the control and drought treatments (Fig. 5b). After re-watering, the total NSC pool size further decreased, and a significant difference was detected between the CK-CK and drought-CK treatments (P < 0.05). NSC fractions (relative allocation of NSC content) in different tissues/organs varied significantly under the control treatment (P < 0.05) and were found in the following decreasing sequence: BA > ON > CN > CR > FR > SW (Fig. 5c). The pattern of NSC allocation in the drought and drought-CK treatments differed from that in the control treatment. The NSC allocation to below-ground tissues/organs decreased significantly in the drought treatment (Fig. 5c). After re-watering, the NSC allocation to below-ground tissues/organs did not recover to the control level. Moreover, the total NSC allocation to the needles decreased (Fig. 5c).

# Discussion

# Differences in NSC concentrations in different tissues/organs

Significant differences were observed in the NSC concentrations among different tissues/organs most likely due to the differences in the physiological functions of each tissue and in the variations in NSC components. Large variations in the NSC concentrations among different tissue types have been reported (Li et al. 2001). Needles are the physical "platform" for photosynthesis, i.e., the manufacture of sugars. Soluble sugars, especially glucose, are the major NSC component in needles. No significant differences in the NSC concentrations were found among needles of saplings in different age classes. This result is contrary to the report of Li et al. (2001), who found that current-year needles display significantly lower NSC

 Table 1
 F-statistics and probabilities (P) from two-way analysis of variance on soluble sugar, starch, non-structural carbohydrate after drought treatment in Chinese fir (Cunninghamia lanceolata) saplings

Variables	Soluble sugar		Starch		NSC	
	F	Р	F	Р	F	Р
Drought treatment	1.425	0.240	71.560	< 0.001	34.865	< 0.001
Tissues/organs	87.838	< 0.001	13.144	< 0.001	62.491	< 0.001
Treatment × tissues/organs	9.544	< 0.001	5.230	0.001	4.726	0.002



**Fig. 4** Responses of soluble sugar (**a**), starch (**b**), and total NSC concentrations (**c**) in different tissues/organs of Chinese fir saplings to drought recovery treatments (September 10, 2013). Values are mean  $\pm$  SE (n = 4). The *asterisks* indicate the significant differences between the control (CK–CK) and drought recovery (drought-CK) treatments in individual tissue. \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001. *CN* current-year needles, *ON* one-year-old needles, *BA* barks, *SW* stemwoods, *CR* coarse roots, *FR* fine roots

concentrations than the two-year-old needles of Pinus cembra. This discrepancy may largely be attributed to the differences between the plants used. We used saplings, whereas Li et al. (2001) used adult trees. The effect of needle age on needle traits depends on the light availability in the canopy of adult trees (Eimil-Fraga et al. 2015). In our study, a lack of significant differences in the NSC concentrations among needles of saplings in different age classes is most likely due to the similar light availability for saplings, especially those grown in pots. The NSC concentrations were significantly higher in the bark or phloem of P. cembra (Li et al. 2002), Populus tremuloides (Landhäusser and Lieffers 2003), P. cembra (L.), and Larix decidua (Gruber et al. 2013) than those in xylem wood, confirming the significant differences we found in the NSC concentrations in barks and sapwoods. Zhang et al. (2014) also reported that the NSC concentrations were significantly higher in the bark than in the woods of 12 temperate tree species. The higher NSC concentrations in the bark than in xylem wood can be ascribed to the high metabolic activity of the barks and to their function in long-distance transport of carbohydrates, as indicated by the high sucrose concentration in the present study. Roots, especially CR, are important storage reserve of NSC. Such function reflects the buffering capacity of trees against various stresses. This characteristic might have caused higher NSC concentrations in CR than in FR as revealed in our study. Fan and Guo (2010) reported that the NSC concentrations were significantly higher in higher-order roots (large diameter) than in lower-order roots (small diameter); these results are similar to ours in this study.

#### Response of NSC to drought and re-watering

The responses of NSC and its components to drought varied among the different tissues/organs. The total NSC concentrations in the needles decreased under drought. Coincidentally, the soluble sugar concentration increased or remained unchanged, whereas starch concentration decreased. This outcome is consistent with previous results obtained (Lee et al. 2008; Hartmann et al. 2013a). Similar to needles, the bark demonstrated increased soluble sugar concentrations at the expense of starch under drought

**Table 2** F-statistics and probabilities (P) from two-way analysis of variance on soluble sugar, starch, non-structural carbohydrate after rewatering treatment in Chinese fir (Cunninghamia lanceolata) saplings

Variables	Soluble sugar		Starch		NSC	
	$\overline{F}$	Р	F	Р	F	Р
Re-watering treatment	156.189	< 0.001	49.296	< 0.001	216.492	< 0.001
Tissues/organs	24.135	< 0.001	15.441	< 0.001	29.182	< 0.001
Treatment $\times$ tissues/organs	14.732	< 0.001	10.514	< 0.001	15.478	< 0.001



Fig. 5 Responses of the biomass (a), total NSC pool size (b) and NSC fraction (c) to drought and re-watering. NSC fraction indicates the relative allocation of NSC content to different tissues/organs. Values are mean  $\pm$  SE (n = 4). The *lowercase letters* indicate significant differences in the different treatments (P < 0.05)

treatment. This phenomenon may largely be explained by the mechanism of osmotic adjustment to increase drought tolerance (Wang and Stutte 1992) because monosaccharides, being the most important osmoticum for adjustment, can significantly increase the cell osmotic pressure. Our work did not find a reduction but rather detected a significant increase in the total NSC concentrations in SW under drought treatment. Moreover, SW demonstrated greater responses to drought than BA. This finding is contrary to the view that phloem or bark demonstrates high activity and that their carbohydrate availability may reflect the altered carbon sink-source balance under stress. Recent studies have focused on the difference in NSC reserves between phloem and xylem (Anderegg et al. 2012; Landhäusser and Lieffers 2003; Li et al. 2002; Gruber et al. 2013), whereas little attention has been given to the response of phloem and xylem NSC contents to drought. Therefore, the underlying response mechanisms must be investigated further. The total NSC concentration in the roots under drought treatment declined more significantly than in the above-ground tissues/organs, with a reduction of approximately 56 %. Similar to our findings, recent results have shown that severe drought decreases root NSC concentrations (Hartmann et al. 2013a, b). Moreover, drought is likely to reduce carbon assimilation (Brodribb and McAdam 2011) and impede carbon translocation (Hartmann et al. 2013a, b). This phenomenon explains the progressive depletion of in situ carbon reserves in the roots observed in the present study, as indicated by the concurrent pronounced decrease in starch and soluble sugar concentrations.

Sapling death occurred in the drought-CK treatment as confirmed by the result of needle staining. The outcome was further supported by the failure of the total NSC concentration in the drought-CK treatment to recover after re-watering. Interestingly, we found that NSC concentration significantly declined both in the roots and aboveground tissues/organs, except in SW, after re-watering. However, this effect was less prominent in the aboveground tissues/organs than in the roots, as indicated by the significant interactive effect of drought and tissues/ organs on NSC concentration. Hartmann et al. (2013a, b) found a different response of NSCs in the above-ground tissues/organs and roots of Norway spruce saplings to lethal drought, and their finding is consistent with our results. They suggested that declining plant water potential caused by drought can result in xylem cavitation and failure in phloem function (carbon translocation), thereby causing local depletion of root carbon reserves. The current study did not measure the predawn leaf water potential, phloem turgor pressure, and xylem cavitation. Therefore, testing the carbon translocation failure from the above-ground tissues/organs to the roots was impossible. Carbon starvation occurs when the supply of available carbohydrate falls below metabolic requirements, which results from stomatal closure to avoid water deficiency after extreme drought (McDowell et al. 2008, Sevanto et al. 2014). Our study found that the NSC concentration considerably declined in the roots and either increased or remained the same in the above-ground tissues/organs during drought treatment. Moreover, the NSC concentration both in the above-ground tissues/organs and roots did not return to the control level after re-watering.

Our results also indicated that carbon starvation may occur only in the roots and not in the above-ground tissues/organs. However, mortality of Chinese fir sapling is not only caused by carbon starvation in roots. Carbohydrate metabolism and plant hydraulics are mutually dependent (McDowell et al. 2011; Anderegg and Callaway 2012; Mitchell et al. 2013; De la Serrana et al. 2015; Nardini et al. 2016). Further studies are required to simultaneously study carbohydrate metabolism and plant hydraulics in the above- and below-ground tissues/organs in response to drought (Hartmann et al. 2013a).

# Conclusions

The differences in the physiological functions of each tissues/organs caused significant differences in the NSC concentrations in various tissues/organs. We confirmed the effect of extreme drought on the varying NSC concentrations in the above- and below-ground tissues/organs. The NSC concentration significantly declined both in the above- and below-ground tissues/organs after re-watering, and a more pronounced decline in NSC concentration was observed in the roots. We speculated that the drought-induced failure of the phloem functions resulted in the uncoupling of the root system from the above-ground tissues/organs; thus, the aboveground tissues/organs and the roots responded differently to drought stress. However, the degree by which the results we obtained from the saplings will apply to mature trees is unknown. Further studies are needed to validate the coupling of the above- and belowground tissues/organs and the response of this coupling to extreme drought in natural ecosystems or field experiments.

Author contribution statement Qingpeng Yang participated in the experimental design, data analyses, and writing of the paper. Weidong Zhang participated in statistical analyses, discussion, and writing of the paper. Renshan Li carried out most of the experiments and participated in data analyses. Ming Xu participated in the experimental design and revised the manuscript. Silong Wang designed and directed the study.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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