Drought stress introduces growth, physiological traits and ecological stoichiometry changes in two contrasting *Cunninghamia lanceolata* cultivars planted in continuous-plantation soils

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Research Article

Keywords: *Cunninghamia lanceolata*, drought stress, physiological traits, ecological stoichiometry, continuous-plantation soil, super cultivar

Posted Date: April 12th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-393259/v1>

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Version of Record: A version of this preprint was published at BMC Plant Biology on August 18th, 2021. See the published version at <https://doi.org/10.1186/s12870-021-03159-3>.

Abstract

Background: The decrease of *Cunninghamia lanceolata* (Lamb.) production on continually planted soil is an essential problem. In this study, two cultivars (a normal cultivar, NC, and a super cultivar, SC) with two-year-old seedlings were grown in two types of soil (non-planting of Chinese fir, NP soil; continuous planting of Chinese fir, CP soil) with three watering regimes, and the interaction effects on plant growth and physiological traits were investigated.

Results: The water contents of normal water control (CK), medium water content (MWC) and low water content (LWC) soil reached 75%-80%, 45%-50% and 20%-25%, respectively, of the field water capacity. The results indicated that both CP soil and LWC soil had negative effects on growth and physiological traits. In both cultivars, CP soil significantly decreased plant growth and the performance of physiological traits. The LWC soil changed the ecological stoichiometry in the three organs, induced oxidative stress, promoted water use efficiency and damaged chloroplast ultrastructure. Compared with NC, the SC cultivar was more tolerant to CP soil and drought stress.

Conclusions: The CP soil shows negative effect on *C. lanceolata*'s physiological traits, and these effects can be exacerbated by drought stress. Therefore, the utilization of continuous planted soil can cultivate improved varieties of *C. lanceolata* and maintain water capacity. This can improve their growth and physiological performance to a certain extent.

Background

Plantation forests have increased global forest cover. However, there is ample evidence for the negative effect of plantation forests on ecosystem functions [1–3]. One of the major problems is that forest plantations generally show degraded soil qualities after long-term successive planting, with lowered N availability to plants [4]. Indeed, effects of continuous-plantation soil on the soil microbial community structure could affect soil quality and productivity [5]. Because of this, the dominant tree height has declined by 7%–23% in continuous-plantation soils of Chinese fir plantations [6]. In addition, drought is becoming more serious in many regions [7, 8]. Drought-stressed plants showed lower gas exchange rates, up-regulation of antioxidant enzymatic systems as well as accumulation of osmolytes [9, 10]. And the utilization of fertilizers were affected by the water content in soil [11]. In nature, plants are generally exposed to a combination of drought and degraded soil. The responses of plants to the interaction of environmental stresses cannot be the same as the responses of plants to each individual stress [12]. Therefore, studies of the combination of stresses are of considerable significance.

The decline of productivity in continuous cropping Chinese fir ecosystems has caused much concern and is a crucial problem that needs to be solved. Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) is an endemic, evergreen coniferous species that is cultivated as a commercial tree. Due to the advantageous features such as fast growth, pest and disease resistance, and timber quality, it is one of the most important timber tree species in the tropics and subtropics of China. Chinese fir plantations have been widely implemented in South China [13]. Continuous cropping of Chinese fir has caused soil degradation resulting in reduced productivity [14–16]. Some researchers, however, have reported that soil fertility did not necessarily decrease with plantation development. Wei et al. [17] showed that soils under continuous cropping of the third rotation had higher soil fertility than those of the second rotation. Consequently, we do not know yet whether soil degradation is a universal problem for continuous cropping.

In this study, two cultivars (a normal cultivar, NC and a super cultivar, SC) with two-year-old seedlings of *C. lanceolata* were grown in two soil types (soil from the land with non-planting of Chinese fir, NP soil; soil from the land with continuous planting of Chinese fir, CP soil) with three watering regimes. We investigated the cultivar-specific responses of *C. lanceolata* in regard to growth, gas exchange, photosynthetic pigments, osmotic adjustment, reactive oxygen species (ROS), enzymatic antioxidants and ultrastructural integrity under CP soil, drought, and their combination. Here, we addressed the hypotheses that growth and physiological traits would be decreased by consecutive monoculture in Chinese fir plantations. We also tested the hypothesis that the super cultivar would be less affected by drought and continuous planting soil than the normal cultivar. Moreover, we proposed that an interaction between drought and continuous planting soil would be present. More specifically, we aimed (1) to determine whether and which growth and physiological traits of *C. lanceolata* are affected by exposure to continuous planting soil, drought and their combination, and (2) to evaluate whether the water condition can promote the effect of continuous planting soil.

Results

Water, cultivar and CP soil effects on biomass accumulation and chlorophyll content

Overall, *C. lanceolata* in the soil without planting (NP) showed higher increments of height (HI), basal diameter (DI) and dry mass allocation (DMA) than plants grown in the soil with continuous planting of *C. lanceolata* (CP) (Table 1). In the two cultivars, HI, DI and DMA showed a significant decrease in response to water stress and CP soil alone, and a much more dramatic decrease occurred when LWC and CP were applied together. The R/S ratio was affected by the interactions of cultivar and CP soil, showing that *C. lanceolata* under LWC had a lower R/S ratio than under MWC and CK. In both cultivars, LWC significantly increased total chlorophyll content and carotenoid content in the two soil types. The chlorophyll a/b ratio was unaffected by either cultivar or CP soil, while water stress significantly increased the chlorophyll a/b ratio.

Table 1

Height increment (HI), basal diameter increment (DI), dry mass allocation (DMA), root to stem ratio (R/S), total chlorophyll content (TChl), chlorophyll a/b ratio (Chl_{a/b}), carotenoid content (Caro) in two cultivars with two-year-old seedlings of *C.lanceolata* as affected by water, soil and their interaction

Cultivars	Water condition	Soil	HI(g)	DI (mm)	DMA (g)	R/S	TChl	Chl _{a/b}	Caro
NC	CK	NP	26.03 ± 1.64a	5.42 ± 0.75ab	33.20 ± 4.13b	0.43 ± 0.06ab	0.96 ± 0.08d	2.45 ± 0.03bc	0.23 ± 0.00d
NC	CK	CP	16.13 ± 1.87de	3.34 ± 0.38e	14.29 ± 0.83e	0.41 ± 0.06b	0.92 ± 0.03d	2.47 ± 0.05b	0.22 ± 0.02d
NC	MWC	NP	19.75 ± 1.54cd	4.26 ± 0.61cd	19.23 ± 1.97d	0.44 ± 0.05ab	1.53 ± 0.00c	2.81 ± 0.06a	0.31 ± 0.06bc
NC	MWC	CP	11.79 ± 1.32f	3.10 ± 0.42e	9.15 ± 1.32g	0.49 ± 0.01a	1.60 ± 0.19c	2.85 ± 0.13a	0.28 ± 0.02bc
NC	LWC	NP	14.49 ± 1.31ef	3.72 ± 0.36d	11.92 ± 1.69f	0.30 ± 0.02cd	2.68 ± 0.34a	2.79 ± 0.17a	0.40 ± 0.02a
NC	LWC	CP	8.08 ± 1.03g	3.11 ± 0.6e	7.63 ± 0.8h	0.28 ± 0.03d	2.36 ± 0.17b	2.76 ± 0.02a	0.32 ± 0.05bc
SC	CK	NP	25.38 ± 1.35ab	5.59 ± 0.87a	41.42 ± 4.29a	0.40 ± 0.05b	1.06 ± 0.01d	2.22 ± 0.16c	0.25 ± 0.01cd
SC	CK	CP	21.98 ± 1.93bc	4.78 ± 0.44bc	25.25 ± 2.65c	0.46 ± 0.05ab	0.85 ± 0.00d	2.40 ± 0.05bc	0.24 ± 0.02d
SC	MWC	NP	20.22 ± 1.63cd	5.05 ± 0.22ab	28.43 ± 1.28bc	0.34 ± 0.04c	1.50 ± 0.04c	2.75 ± 0.13a	0.29 ± 0.02bc
SC	MWC	CP	17.92 ± 1.49de	4.77 ± 0.58bc	17.63 ± 1.51d	0.44 ± 0.05ab	1.68 ± 0.15c	2.72 ± 0.11a	0.30 ± 0.03bc
SC	LWC	NP	15.38 ± 1.28ef	4.73 ± 0.48bc	18.73 ± 1.29d	0.27 ± 0.02d	2.72 ± 0.09a	2.57 ± 0.16ab	0.33 ± 0.04ab
SC	LWC	CP	7.60 ± 1.02g	3.59 ± 0.27de	8.37 ± 0.99gh	0.34 ± 0.03c	1.71 ± 0.02c	2.75 ± 0.04a	0.35 ± 0.11ab
		P:Fw	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.001	0.0004	< 0.0001
		P:Fc	0.0013	< 0.0001	< 0.0001	0.2782	0.0671	0.5350	0.9392

Values are mean ± SE. Values followed by different small letters and within parameters are significantly different at the $P < 0.05$ level according to Tukey's test. NC, normal cultivar; SC, super cultivar; CK, control watering regime; MWC, mild water stress; LWC, heavy water stress; Fw, water stress effect; Fc, cultivar effect; Fs, soil effect; Fw× Fc, water and cultivar interaction; Fs× Fc, soil and cultivar interaction; Fw× Fs, water and soil interaction; Fw× Fc× Fc, soil, water and cultivar interaction.

Cultivars	Water condition	Soil	HI(g)	DI (mm)	DMA (g)	R/S	TChl	Chl _{a/b}	Caro
		P:Fs	< 0.0001	< .0001	< 0.0001	0.0380	0.0197	0.1746	0.2561
		P:Fw× Fc	0.7955	0.2412	0.0373	0.0623	0.0086	0.8434	0.4706
		P:Fw×Fs	0.6754	0.0885	< 0.0001	0.3968	0.0013	0.3346	0.7508
		P:Fc×Fs	0.0031	0.0569	0.4922	0.0365	< 0.0001	0.0769	0.0810
		P:Fw×Fc×Fs	0.4997	0.0269	0.1861	0.9037	< 0.0001	0.2423	0.73892
Values are mean ± SE. Values followed by different small letters and within parameters are significantly different at the $P < 0.05$ level according to Tukey's test. NC, normal cultivar; SC, super cultivar; CK, control watering regime; MWC, mild water stress; LWC, heavy water stress; Fw, water stress effect; Fc, cultivar effect; Fs, soil effect; Fw× Fc, water and cultivar interaction; Fc× Fs, soil and cultivar interaction; Fw× Fs, water and soil interaction; Fw× Fc× Fs, soil, water and cultivar interaction.									

Water, cultivar and CP soil effects on P_n , g_s , C_i , E and PNUE

In the two cultivars, P_n , g_s , E and PNUE were all significantly decreased when the seedlings were exposed to MWC or LWC (Table 2). Compared with NP, the seedlings of both cultivars in CP had lower P_n , E and PNUE values and lower C_i values. All these parameters were significantly affected by the interaction between water and soil type.

Table 2

Net photosynthesis rate (P_n), stomatal conductance (g_s), transpiration (E), photosynthetic N use efficiency (PNUE), intercellular carbon dioxide concentration (C_i) in two cultivars with two-year-old seedlings of *C. lanceolata* as affected by water, soil and their interaction

Cultivars	Water condition	Soil	$P_n(\mu\text{mol m}^{-2} \text{s}^{-1})$	$g_s(\text{mol m}^{-2} \text{s}^{-1})$	$C_i(\mu\text{mol mol}^{-1})$	$E(\text{mmol m}^{-2} \text{s}^{-1})$	PNUE ($\mu\text{mol g}^{-1} \text{s}^{-1}$)
NC	CK	NP	4.38 ± 0.44a	0.09 ± 0.03b	267.06 ± 20.67c	0.88 ± 0.11ab	4.52 ± 0.45a
NC	CK	CP	3.02 ± 0.45b	0.12 ± 0.01a	320.06 ± 7.39a	0.73 ± 0.09c	4.28 ± 0.62a
NC	MWC	NP	2.97 ± 0.29b	0.06 ± 0.01c	298.63 ± 5.61b	0.77 ± 0.10bc	2.97 ± 0.28cd
NC	MWC	CP	1.80 ± 0.24f	0.05 ± 0.01d	330.50 ± 14.37a	0.58 ± 0.09d	2.18 ± 0.29e
NC	LWC	NP	2.25 ± 0.12de	0.03 ± 0.01e	165.32 ± 2.81f	0.34 ± 0.03e	2.70 ± 0.14cd
NC	LWC	CP	1.69 ± 0.22f	0.02 ± 0.00f	265.73 ± 21.75c	0.17 ± 0.05f	2.24 ± 0.30e
SC	CK	NP	4.53 ± 0.74a	0.10 ± 0.02b	285.85 ± 27.63b	0.93 ± 0.05a	4.17 ± 0.68ab
SC	CK	CP	2.99 ± 0.40b	0.10 ± 0.03ab	327.32 ± 7.07a	0.85 ± 0.18a	3.09 ± 0.41c
SC	MWC	NP	2.50 ± 0.18cd	0.06 ± 0.01c	294.12 ± 1.62b	0.54 ± 0.04d	2.63 ± 0.19d
SC	MWC	CP	2.80 ± 0.28bc	0.06 ± 0.01cd	293.37 ± 2.94b	0.55 ± 0.04d	3.82 ± 0.38b
SC	LWC	NP	2.29 ± 0.35d	0.03 ± 0.00e	214.71 ± 14.39e	0.23 ± 0.05f	2.77 ± 0.41cd
SC	LWC	CP	1.94 ± 0.10ef	0.03 ± 0.00ef	251.94 ± 22.11d	0.22 ± 0.06f	2.62 ± 0.14d
		P:Fw	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		P:Fc	0.0188	0.6076	0.2204	0.4886	0.46
		P:F _s	< 0.001	0.4783	0.0019	< 0.0019	< 0.0001
		P:Fw × Fc	0.2662	0.2611	< 0.0001	< 0.0001	< 0.0001
		P:Fw × F _s	< 0.001	< 0.0001	< 0.0001	0.0010	< 0.0001
		P:Fc × F _s	0.00098	0.94	< 0.0001	0.05	0.0022
		P:Fw × Fc × F _s	< 0.001	0.0798	< 0.0001	< 0.0001	< 0.0001
Values are mean ± SE. Values followed by different small letters and within parameter are significantly different at the $P < 0.05$ level according to Tukey's test.							

Water, cultivar and CP soil effects on WUEi, Wp, $\delta^{13}\text{C}$, CUE, NUE and PUE

In the two cultivars, LWC significantly increased WUEi, Wp, $\delta^{13}\text{C}$, CUE, NUE and PUE in *C. lanceolata* planted in the two types soil (Table 3). In addition, in the normal cultivar, seedlings exposed to mild water stress had lower WUEi, $\delta^{13}\text{C}$, CUE, NUE and PUE than those of plants under CK, but this was not the case in the super cultivar. Significant water $\times$ cultivar $\times$ soil interaction effects were detected in WUEi, Wp, $\delta^{13}\text{C}$, NUE and PUE.

Table 3

Instant use efficiency (WUEi), water potential (Wp), foliar carbon isotope composition (δC^{13}), carbon use efficiency (CUE), nitrogen use efficiency (NUE), phosphorus use efficiency (PUE) in two cultivars with two-year-old seedlings of *C. lanceolata* as affected by water, soil and their interaction

Cultivars	Water condition	Soil	WUEi	Wp (MPa)	δC^{13}	CUE	NUE	PUE
NC	CK	NP	4.98 ± 0.70d	-0.86 ± 0.05d	-28.33 ± 0.15c	2.45 ± 0.28de	3.06 ± 0.07de	2.37 ± 0.16e
NC	CK	CP	4.13 ± 0.94e	-0.84 ± 0.10d	-28.52 ± 0.23c	2.68 ± 0.31cd	3.04 ± 0.06de	2.71 ± 0.36d
NC	MWC	NP	3.86 ± 0.15ef	-0.89 ± 0.10d	-28.97 ± 0.52d	2.23 ± 0.12de	2.98 ± 0.02e	2.66 ± 0.31de
NC	MWC	CP	3.24 ± 0.86f	-1.26 ± 0.05bc	-28.70 ± 0.29cd	2.18 ± 0.04e	2.40 ± 0.02g	2.39 ± 0.31de
NC	LWC	NP	6.67 ± 0.73c	-1.17 ± 0.16c	-27.25 ± 0.13a	3.00 ± 0.22bc	3.47 ± 0.14bc	3.07 ± 0.36cd
NC	LWC	CP	10.61 ± 1.04a	-1.63 ± 0.18a	-27.78 ± 0.18b	3.90 ± 0.28a	3.51 ± 0.09b	4.60 ± 0.77a
SC	CK	NP	4.91 ± 0.50d	-0.96 ± 0.09d	-28.57 ± 0.08cd	2.55 ± 0.20cde	3.12 ± 0.03d	3.15 ± 0.44c
SC	CK	CP	3.58 ± 0.42f	-0.97 ± 0.13d	-28.70 ± 0.30cd	2.22 ± 0.06de	2.76 ± 0.00ef	2.36 ± 0.30de
SC	MWC	NP	4.65 ± 0.18de	-0.96 ± 0.05d	-28.55 ± 0.39cd	2.98 ± 0.19bc	3.23 ± 0.24cd	3.51 ± 0.46bc
SC	MWC	CP	5.09 ± 0.12d	-1.33 ± 0.08bc	-28.26 ± 0.22c	2.33 ± 0.21de	2.54 ± 0.11fg	2.04 ± 0.13e
SC	LWC	NP	10.15 ± 0.62ab	-1.40 ± 0.07b	-27.37 ± 0.17a	3.19 ± 0.06b	3.66 ± 0.22b	3.78 ± 0.29b
SC	LWC	CP	9.60 ± 1.28b	-1.75 ± 0.16a	-27.70 ± 0.31b	3.65 ± 0.10a	4.00 ± 0.02a	3.37 ± 0.38bc
		P:Fw	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0002
		P:Fc	0.03	0.6465	0.07	0.6201	0.097	0.003
		P:F _s	0.46	0.0030	0.0006	0.00576	< 0.0001	0.0369
		P:Fw × Fc	0.0008	0.5172	0.0061	0.01	< 0.0001	0.2845
		P:Fw × F _s	< 0.0001	0.7467	0.0.4	0.0184	< 0.0001	0.0167
		P:F _c × F _s	0.04	< 0.0001	0.59	0.0004	0.01	0.0007

Values are mean ± SE. Values followed by different small letters and within parameters are significantly different at the $P < 0.05$ level according to Tukey's test.

Cultivars	Water condition	Soil	WUEi	Wp (MPa)	δC^{13}	CUE	NUE	PUE
		P:Fw×Fc×Fs	< 0.0001	0.0001	0.0267	0.2484	0.0005	0.3965
Values are mean ± SE. Values followed by different small letters and within parameters are significantly different at the $P < 0.05$ level according to Tukey's test.								

Water, cultivar and CP soil effects on C:N ratio and N:P ratio in leaves, stems and roots

In the normal cultivar, the C:N ratios of roots and stems planted on CP soil were significantly decreased under water stress (Table 4). In addition, soil type affected the C:N ratios of roots, stems and leaves. This showed that the C:N ratios of stems and leaves were higher under CP compared with those of plants under NP. Compared with leaves, the C:N ratios of stems and roots were higher under all treatments. Regarding the N:P ratio, significant increases induced by CP were detected in all organs in both cultivars (Table 4). In both cultivars, there were significant increments in the N:P ratio of roots and leaves when exposed to MWC and LWC. Soil type had a significant effect on the N:P ratios of all organs.

Table 4

Effects of water stress on stoichiometry changes in *C. lanceolata* planted on different continuous plantation soils

Cultivars	Water condition	Soil	Root		Stem		Leaf	
			C:N	N:P	C:N	N:P	C:N	N:P
NC	CK	NP	61.47 ± 4.53ab	4.18 ± 0.08f	75.21 ± 1.15bc	4.45 ± 0.21e	36.75 ± 0.81cd	5.11 ± 1.14e
NC	CK	CP	59.38 ± 6.36ab	8.82 ± 0.11cde	92.00 ± 8.75ab	8.19 ± 0.19c	38.38 ± 2.88c	9.22 ± 1.39de
NC	MWC	NP	53.72 ± 0.78ab	5.67 ± 0.04f	62.95 ± 0.31c	4.40 ± 1.30e	31.86 ± 0.92d	9.47 ± 0.82de
NC	MWC	CP	57.29 ± 5.52ab	17.60 ± 1.20a	81.09 ± 0.94b	12.44 ± 1.60a	40.82 ± 4.08bc	18.43 ± 0.80a
NC	LWC	NP	49.25 ± 3.03b	7.03 ± 0.28ef	62.88 ± 1.53c	5.51 ± 0.17de	35.30 ± 2.85cd	9.51 ± 0.58de
NC	LWC	CP	51.40 ± 0.42b	15.14 ± 0.02ab	82.93 ± 3.64ab	10.92 ± 0.14ab	49.39 ± 1.82a	10.60 ± 5.08bc
SC	CK	NP	57.07 ± 6.50ab	5.51 ± 0.16f	77.03 ± 0.32bc	4.86 ± 0.19de	34.90 ± 1.22cd	5.99 ± 1.99e
SC	CK	CP	66.49 ± 4.76a	10.93 ± 1.18de	87.52 ± 4.77ab	9.47 ± 0.68bc	39.06 ± 0.77bc	15.02 ± 0.33b
SC	MWC	NP	58.71 ± 4.61ab	5.84 ± 0.72f	61.98 ± 2.13c	6.22 ± 0.20d	35.26 ± 1.63cd	9.83 ± 0.14cde
SC	MWC	CP	66.27 ± 7.35a	11.98 ± 1.17bcd	99.19 ± 7.85a	10.14 ± 0.90b	44.81 ± 0.57ab	18.49 ± 1.87a
SC	LWC	NP	49.86 ± 4.75b	7.59 ± 0.26de	80.69 ± 17.77b	5.80 ± 0.09de	45.07 ± 5.77ab	6.89 ± 1.32e
SC	LWC	CP	66.99 ± 3.15a	12.77 ± 0.51bc	83.51 ± 12.48ab	9.73 ± 0.48bc	50.25 ± 2.95a	12.92 ± 4.67cd
		P:Fw	0.0765	0.0155	0.1447	0.0020	0.0001	< 0.0001
		P:Fc	0.0295	0.4654	0.0718	0.8546	0.0239	0.4864
		P:F _s	0.0150	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		P:Fw × Fc	0.4377	0.1390	0.4152	0.1867	0.1135	0.0029
		P:Fw × F _s	0.5494	0.1927	0.1209	0.0632	0.0455	0.0041
		P:F _c × F _s	0.0409	0.1443	0.9489	0.0180	0.3911	0.1370
		P:Fw × Fc × F _s	0.5990	0.3173	0.0863	0.0138	0.1117	0.1373
Values are mean ± SE. Values followed by different small letters and within parameter are significantly different at the <i>P</i> < 0.05 level according to Tukey's test.								

Water, cultivar and CP soil effects on enzyme activity

Compared with CK, APX and POD activity significantly increased as a response to LWC. In contrast, SOD increased only in the super cultivar under CP soil (Fig. 1). In addition, *C. lanceolata* under MWC had higher SOD activity than those of plants under CK and LWC.

Water, cultivar and CP soil effects on soluble protein content and proline content

The seedlings planted in NP showed higher soluble protein content than those of plants under CP, the same as for proline content (Fig. 2). In both cultivars, LWC had increased soluble protein content and proline content. In addition, there was little difference between the two cultivars.

Water, Cultivar And CP Soil Effects On Mesophyll Cells

C. lanceolata had smooth, clean and continuous cell membranes and cell walls in CK and MWC (Fig. 3a–d, g–j). However, alterations in the ultrastructure of Chinese fir leaves under different treatments were detected by the TEM analysis. Compared with the leaves under NP, the leaves under CP had more starch granules. The LWC alone seriously affected chloroplasts in both cultivars under the two types soil, especially for NC-CP-LWC (Fig. 3. f) and for SC-NP-LWC (Fig. 3. k).

Discussion

Growth, photosynthesis and chlorophyll content physiological responses as affected by drought for seedlings planted in continuous-plantation soil

This study examined the hypotheses that growth and physiological responses would be significantly degenerated by consecutive monoculture in *C. lanceolata* plantation soils. In our study, CP soil caused a decrease in height increment, basal diameter increment, dry matter accumulation (DMA), net photosynthetic rate (Pn) and photosynthetic nitrogen use efficiency (PNUE). Similarly, previous studies have reported that productivity declines were observed in long-term monoculture Chinese fir plantations [15, 18]. The CP soil suppressed growth and leaf photosynthesis, as shown in other studies [19], which might be due to three reasons: depletion of nutrient elements, soil acidification and imbalance of soil microbes [14, 20, 21]. Generally, soil degradation in the continuous cropping system of our study appeared to be less serious than expected based on previous reports. In the third rotation, available P showed a tendency to decrease compared with the first rotation. This implies that there may be other factors contributing to the observed decline in growth. For example, acidification could be a problem for continuous cropping ecosystems for *C. lanceolata* growth [22]. Soil acidification and available P deficiency appear to be two of the important factors leading to production decline of *C. lanceolata* in continuous rotation practices.

Pant biomass decreased significantly in the two cultivars affected by drought stress, similar to the results of previous studies reported for other plants species [23, 24]. The effect might be due to decreases in photosynthesis and chlorophyll content [25]. Similarly, the decrease in the contents of C in the leaves under drought conditions showed that plant growth was affected by a low net photosynthetic rate. Drought not only affected the reduction of biomass productivity, but also significantly reduced plant physiological traits such as gas exchange parameters, which have been reported in many previous studies [26, 27]. In this study, drought stress increased WUE_i and $\delta^{13}C$. The production and accumulation of soluble protein in leaves may be caused by nitrogen reserves affected by stress. Compared with the normal cultivar, the super cultivar exhibited higher biomass production and greater water use efficiency when affected by drought, indicating that the super cultivar is more tolerate to drought. Long-term nitrogen use efficiency (NUE) can be estimated using C:N ratio [28]. The observed decrease in the C:N ratio under

drought and CP soil indicate that the combinations of two stresses reduced NUE, which was mainly caused by the decrease of C content and the increase of N content.

Cellular damage and enzyme activities

Drought may enhance the scavenging capacity of reactive oxygen species [29]. Drought can trigger membrane damage. SOD, APX and POD are the components of the plant antioxidative defense system. Our results show that seedlings under drought conditions showed high levels of APX and POD antioxidant enzymes. Similarly, the activities of several antioxidant enzymes, such as APX and SOD, were found to increase under the effects of drought [30, 31]. Proline accumulation is generally observed in plants exposed to drought stress [32]. Soluble protein and free proline contents were significantly increased by drought in both cultivars (Fig. 2); this could contribute to osmotic adjustment. Affected by drought, the long-term water use efficiency evaluated by $\delta^{13}\text{C}$ changed obviously, and the $\delta^{13}\text{C}$ of super cultivar was significantly higher than that of the normal cultivar. Compared to the normal cultivar, the super cultivar showed higher dry matter accumulation and accumulated much more free proline for osmotic adjustment. The super cultivar also showed a more efficient antioxidant system, with higher activities of SOD than the normal cultivar. According to the effects of drought on the growth parameters, free proline and WUEi, it was considered that super cultivar had higher drought tolerance than the NC.

The previous results showing that the mesophyll cell ultrastructure of both cultivars shows damage to the nuclear envelope and the membranes of mitochondria, thylakoids and stromata under drought treatment, and in consist with our result. And this effect was more obviously in CP soils. These breakdowns in cell membranes may be due to an increase in the reactive oxygen species (ROS) accumulation in different cell compartments, since the ROS have an important role in the inner membrane system organization.

Growth and physiological responses as affected by the combination of drought and CP soil

In field conditions, plants are often affected by many different abiotic stress factors simultaneously. Plant responses to different combinations of stresses are unique [33]. This study investigated the interaction of drought and CP soil on growth and physiological traits. The plant height increment and biomass production under the two stress combinations were more severely affected than single drought. And when CP soil was combined with drought, the drought-induced reduction was most obvious. Moreover, the intensive damage in ultrastructure caused by drought $\times$ CP confirmed that the effects of antioxidation in CP soil were modified by the water supply. When plants grew in CP soil, there was little difference in plant growth, POD activity and free proline content under water sufficiency and drought stress, which indicated that CP soil may aggravate certain effects under water stress conditions.

Conclusions

LWC and CP soil treatment caused damage to plant growth, photosynthesis and other physiological indicates, as well as to the ultrastructure of mesophyll cells. We suggest that the super cultivar is better adapted to drought and CP soil than the normal cultivar. In contrast, distinctly higher biomass and APX activity and proline levels were observed in the super cultivar when compared to the normal cultivar. Our results suggest that the super cultivar exhibits greater tolerance to drought and CP soil than the normal cultivar. These results suggest that low water content aggravates the CP soil stress impact on *C. lanceolata* seedlings.

Methods

Plant materials and experimental design

The two-year-old seedlings were from Yangkou Forest Farm, Fujian Province. The normal cultivar were the first generation seedlings, and the super cultivar were the third generation varieties. The seedlings with relatively consistent height and diameter were planted in 30-L large plastic pots. Seedlings were grown in a controlled environment room at Zhejiang A & F University (N30°23', E119°72'), China. Before the trial started, to keep the seedlings growing well, we irrigated the pots once every three days or when there was a need.

The experiment employed a completely randomized design with 12 factorial combinations of two Chinese fir cultivars (NC and SC), two types of soil (NP soil and CP soil), and three levels of water stress. This trial had three replications per treatment and five plants per replication (plastic pot). The Chinese fir cultivars were collected from Shaxian, Fujian province. SC and NC were 2.5 and 1.5 strain cultivar of *C. lanceolata*, respectively. SC has stronger ability of height growth and diameter growth than NC [34]. The sapling stage before available for timber production is shorter than that of NC. The performance of SC in clear-cutting forestland of Chinese fir plantation was significantly better than that of sprouting trees [35]. However, the ability to adapt to drought stress, and their performance in logging area were less studied. The NP soil was the soil from forest lands without planted Chinese fir, and most of the trees in this forest land were subtropical species of evergreen broad-leaved trees. The CP soil was the soil from a Chinese fir forest that had been replanted more than 20 years after the first fir harvest. The properties of the non-continuous planting soil (NP soil) used in this study were as follows (based on kg⁻¹ dry soil): pH 5.47, total N 1.21 g, hydrolysable N 162.07 mg, total phosphorus 0.61 g, available phosphorus 2.1 mg, total potassium 13.69 g, organic matter 36.36 g. The properties of continuous planting soil from the Chinese fir forest land (CP soil) were as follows (based on kg⁻¹ dry soil): pH 4.63, total N 0.92 g, hydrolysable N 127.08 mg, total phosphorus 0.43 g, available phosphorus 1.53 mg, total potassium 10.41 g, organic matter 28.22 g.

There were three drought treatments: one was under normal water control (watered and maintained at 75%–80% field water capacity, normal water control, CK); a second treatment was under medium drought stress (watered and maintained at 45%–50% field water capacity, medium water content, MWC), and the third was under heavy draught stress (watered and maintained at 20%–25% field water capacity, low water content, LWC). Water was added when the percentage was outside the specified level at 16:00–18:00 per 3 d by the weight method.

During the executing process, the potted seedlings of each test treatment were periodically rotated to minimize the influence of the growth factors of each treatment. During the last 10 d of this trial, protecting enzyme activity, lipid periodization materials, relative electrolyte conductivity, gas exchange, chlorophyll fluorescence and chlorophyll pigment content were all evaluated. After harvesting, samples were separated into leaves, stems and roots. The samples were used for the measurements of water content, biomass, total carbon (C), total nitrogen (N) and total phosphorus (P) in different organs.

Assays of height growth, biomass and water content

At the beginning, we selected 30 plants per cultivar to measure their height and biomass. The samples were separated into different organs for biomass assays. This was done again at the end of the trial. The increases in biomass, height growth and water content were calculated by subtracting the final data from the mean initial data. Water content was calculated by fresh weight minus the dry weight. The root-to-shoot ratio was calculated by root biomass and shoot biomass. Biomass was obtained by oven drying at 60°C until a constant weight was reached [36].

Assays of nutrient biological stoichiometry, nutrient use efficiency

The contents of C, N and P in roots and leaves were analyzed and determined. The C content was measured by an elemental analyzer (Vario Max cube CNS, Elementar, Germany). The N and P content in different organs were measured by the micro Kjeldhal and molybdenum antimony colorimetric methods, respectively [36, 37]. Nutrient biological stoichiometry in leaf, stem and root were calculated by N content/ P content. C use efficiency (CUE), N use efficiency (NUE) and P use efficiency (PUE) were calculated by the ratios of nutrient content in organs above the soil and in organs below the soil [38]. These were to evaluate the allocation of nutrient in plant.

Determination of enzymes activity and lipid periodization

Fresh leaf samples from the third or fourth expanded leaves were collected for enzymes extraction. The samples were collected and put into boxes with ice bags. Enzymes were extracted at 4°C from approximately 0.2 g leaf samples with 100 mM phosphate buffer (pH 7.8). This buffer contained 0.1 mM MEDTA, 1% (v/v) polyvinyl pyrrolidone (PVP), 0.1 mM phenyl methyl sulphonyl fluoride (PMSF) and 0.2% (v/v) Triton X-100. Extracting solutions were centrifuged at 6,000 r/min for about 30 min. The supernatants were used for the measurements of superoxide dismutase (SOD; EC 1.15.1.1), peroxidase (POD; EC 1.11.1.11) and soluble protein.

SOD activity was assayed by the inhibition of the photochemical reduction of β -nitro blue tetrazolium chloride (NBT) [37, 39], and SOD was measured at 560 nm with a spectrophotometer (Shimadzu UV-2550, Kyoto, Japan). POD activity was measured with guaiacol as the substrate at 470 nm [40]. Malondialdehyde (MDA) content is an essential parameter to illustrate the lipid peroxidation level, and this was determined via the thiobarbituric acid method (TBA) [37]. Total soluble protein content was determined by the Coomassie brilliant blue method [37]. The APX enzyme activity was determined by the ascorbic acid method.

Proline content was determined by the acid ninhydrin occlusion method [41]. To a 0.20 g sample of leaves, 5 ml 3% sulfosalicylic acid solution was added with covering and extracted in a boiling water bath for 15 min (shaking frequently during the extraction), and then filtered in a clean test tube after cooling. The filtrate is the extract of proline. After obtaining the extract, the measurement was carried out with reference to the literature.

Gas exchange

We selected five samples from the third or fourth fully expanded and exposed young leaves for gas exchange and chlorophyll fluorescence measurements.

Gas exchange was measured with the Li-cor 6400XT portable photosynthesis system (Li-cor Biosciences, Inc., Lincoln, USA) on sunny days during 8:30 to 11:00. In this system, the ambient CO₂, light intensity, relative humidity and temperature were controlled at 400 $\mu\text{mol}\cdot\text{mol}^{-1}$, 1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 60% and 28°C, respectively. The CO₂ was provided by a dedicated CO₂ steel cylinder for Li-cor 6400. The light was provided by an LED red-blue light chamber. The leaf in the chamber were collected and the area were measured with leaf area analysis system (CI202, CID, USA).

The photosynthetic N use efficiency (PNUE) was calculated as the ratio between the photosynthetic rate and leaf N concentration per area [29].

Water use efficiency

The instantaneous water use efficiency (WUE_i) was determined by the ratio of maximum carbon assimilation at saturating light (A_{max}) to leaf transpiration rate (E). The five leaf samples per replication were selected for the

measurement of water potential (Wp) with pressure tank (3005, Soilmoisture Equipment Corp, USA). These were done during 2:00–4:30 before sunrise.

Five leaf samples per treatment were selected for the measurement of $\delta^{13}\text{C}$ using an isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher Scientific, Inc., USA), combined with an elemental analyzer (Flash EA1112 HT, Thermo Fisher Scientific, Inc.). The samples were burned at high temperature to generate CO_2 in the elemental analyzer. We measured the ratio of ^{13}C content to ^{12}C content using the mass spectrometer and calculated the ratio of $\delta^{13}\text{C}$ after comparing with the international standard substance (Pee Dee Belnite, PDB).

Chlorophyll content and ultrastructure of chloroplasts

The samples were collected for each treatment for chlorophyll content determination. The harvested fir leaves were quickly washed and dried, and the midrib was cut. The sample was then cut into a 0.2 cm filament or a small piece and evenly mixed. The samples were weighed to the nearest 0.1 g, placed in a test tube, and 8 ml of 95% ethanol was added. The leaves were soaked in the dark for 24 hours, and shaken for three to four times in the middle of the period until they turned white. Each treatment was repeated three times [41].

Five samples per treatment were selected for examining the ultrastructure of chloroplasts using a transmission electron microscope (Hitachi H-7650, Hitachi, Ibaraki, Japan). The samples were processed as follows: (1) Double fixation: The specimen was first fixed with 2.5% glutaraldehyde in phosphate buffer (0.1M, pH7.0) for more than 4 h, washed three times in the phosphate buffer (0.1M, pH7.0) for 15 min at each step, then post fixed with 1% OsO_4 in phosphate buffer (0.1M, pH7.0) for 1–2 h and washed three times in the phosphate buffer (0.1M, pH7.0) for 15 min at each step. (2) Dehydration: The specimen was first dehydrated by a graded series of ethanol (30%, 50%, 70%, 80%, 90%, 95% and 100%) for about 15–20 min at each step, then transferred to absolute acetone for 20 min. (3) Infiltration: The specimen was placed in a 1:1 mixture of absolute acetone, and the final Spurr resin mixture for 1h at room temperature, and then transferred to a 1:3 mixture of absolute acetone and the final resin mixture for 3h and to the final Spurr resin mixture overnight. (4) Embedding and ultrathin sectioning: specimens were placed in Eppendorf tubes containing Spurr resin and heated at 70°C for more than 9h. The specimens were sectioned with a LEICA EM UC7 ultratome, and sections were stained by uranyl acetate and alkaline lead citrate for 5–10 min, respectively, and observed using a Hitachi Model H-7650 TEM.

Statistical Analyses

Data were analyzed by the MANOVA procedure of the Statistic Analysis System (SAS). Fertilizer treatments and water availability were analyzed as main factors. Multiple comparisons were made using Duncan's test at a significance level of $\alpha = 0.05$.

Abbreviations

NC: normal cultivar; SC: super cultivar; NP: from the land without planting *C. lanceolata*; CP: from the land with continues-planting *C. lanceolata*; CK: normal water control; MS: medium stress; HS: heavy stress; CUE: C use efficiency; NUE: N use efficiency; PUE: P use efficiency; SOD: superoxide dismutase; NBT: nitro blue tetrazolium; TBA: thiobarbituric acid; MDA: malondialdehyde; POD: peroxidase; PNUE: photosynthetic N use efficiency; WUE_i : instantaneous water use efficiency; Wp: water potential; DMA: dry matter accumulation; Pn: net photosynthetic rate; HI: Height increment; DI: basal diameter increment; R/S: root to stem ratio; TChl: total chlorophyll content; $\text{Chl}_{a/b}$: chlorophyll a/b ratio; Caro: carotenoid content; E: transpiration; Ci: intercellular carbon dioxide concentration; δC^{13} : foliar carbon isotope composition.

Declarations

Acknowledgements

Not applicable.

Funding

This work was supported by the People's Government of Zhejiang Province-Chinese Academy of Forestry cooperative project (Grant No. 2019SY01), and the Fundamental Research Funds for the Central Non-Profit Research Institution of CAF (CAFYBB2019MB005). All funding agencies provided financial support only and did not participate in the design of experiments, sampling and manuscript writing tasks.

Availability of data and materials

The relevant procedures and information can not be made public for the time being but are available from the corresponding author on reasonable request.

Authors' contributions

Conceptualization: BLD, ZZW and YBZ; Data curation: YFB; Formal analysis: AKW; Funding acquisition: XHD; Investigation: YFB and AKW; Project administration: YKW; Resources: HBZ; Writing: XHD, FYB. All authors had read and approved the final manuscript.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Antioxidant enzyme activities of *C. lanceolata* under different treatments. NC, normal cultivar; SC, super cultivar; CK, control watering regime; MWC, mild water stress; LWC, heavy water stress. Values are mean $\pm$ SE Values followed

by different capital letters are significantly different at the $P < 0.05$ level according to Tukey's test.

Figure 2

Soluble proteins and proline contents of *C. lanceolata* under different treatments. NC, normal cultivar; SC, super cultivar; CK, control watering regime; MWC, mild water stress; LWC, heavy water stress. Values are mean $\pm$ SE. Values followed by different capital letters are significantly different at the $P < 0.05$ level according to Tukey's test.

Figure 3

Transmission electron microscopy observations of mesophyll cells in *C. lanceolata* planted in different continuous plantation soils. NC, normal cultivar; SC, super cultivar; CK, control watering regime; MWC, mild water stress; LWC, heavy water stress. Values are mean $\pm$ SE. Values followed by different capital letters are significantly different at the $P < 0.05$ level according to Tukey's test. NC-CK-NP (a), NC-CK-CP (b), NC-MWC-NP (c), NC-MWC-CP (d), NC-LWC-NP (e), NC-LWC-CP (f), SC-CK-NP (a), SC-CK-CP (b), SC-MWC-NP (c), SC-MWC-CP (d), SC-LWC-NP (e), SC-LWC-CP (f). The bar corresponds to 1 μ m. C, chloroplast; PM, plasma membrane; CW, cell wall; P, plastoglobulus; S, starch granule; N, nucleus; NC, nucleolus; V, vacuole.