Supplemental information for: Multi-walled carbon nanotubes improve nitrogen use efficiency and nutritional quality in *Brassica campestris*

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S1. Determination of Determination of soil physical and chemical properties

(1) Soil pH

10g of air-dried soil were weighed with a 10-mesh sieve in a beaker, added 25ml of distilled water to make the water-soil ratio 1:2.5, intermittently stirred or vibrated for 30min, place it for 30min, and measured it with a pH meter. Three repeated samples were tested for each data point, and the experiment was repeated three times to get the average value.

(2) Soil organic carbon and total nitrogen

30-40mg of air-dried soil were weigh with 100 mesh sieve, pack the sample in a tin boat, measure the content of soil organic carbon by using Vario EL-III element analyzer, and calculate the content of soil organic matter by the following formula. Each treatment is repeated in triplicate to average.

Soil organic matter (g/kg) = soil organic carbon (%)×10×1.724

Total nitrogen (g/kg) = soil organic nitrogen (%)×10

(3) Soil available phosphorus

5.00g of air-dried soil sample were weighed and 25mL of extractant (0.05mol/L HCl-0.025mol/L H_2SO_4) were added, shook for 5min, filtered and took 1mL of filtrate, add 24 ml of working solution (5 ml ASA and 10mL ammonium molybdate, constant volume to 1L), stood still for 30min for color development, and the absorbance was recorded at 700 nm by a N2S spectrophotometer.

(4) Soil alkaline nitrogen

2g of air-dried soil with 20-mesh sieve were weighed and put it in a clean room outside the diffusion dish, and gently shook it to make the soil even and smooth. 2mL of H_3BO_3 indicator were took into the inner chamber of the diffusion dish, rotated several times to make the indicator solution spread out in the inner chamber, 10mL1mol/L NaOH solution were added to completely cover the soil, put the cover tightly and put it in the incubator for 24h, then took it out, titrated with 0.005mol/l H2SO4, and record the titration volume to obtain the content of alkali nitrogen.

(4) Soil available potassium

5.00g of air-dried soil sample with 1mm sieve were weighed and put into a 100 mL centrifugal tube. 50mL of 1mol/L NH_4OAc（pH=7）solution were added into the centrifugal tube, covered it with a plastic film and shook it for 30 min. The filtrate was filtered into a test tube and measured with a flame photometer the galvanometer reading was record.

S2. Determination of soil enzyme activity

(1) Urease activity

5 g of air-dried soil samples were weighed into 100 mL plastic bottles, added 1 mL of toluene was added and the bottles were shaken until thoroughly mixed. After 15 min of mixing, 10 mL of 10% urea solution and 20 mL of citrate buffer solution with pH of 6.0 was added, with shaking and the soil was cultivated in a constant temperature incubator (MGC-350BP, Blue Pard, Shanghai, China) for 24 h at 37 ℃. After culturing, the samples were filtered with qualitative filter paper with a diameter of 11cm (New Star, Hangzhou, China) and 1 mL of filtrate was added into a 50 mL volumetric flask. Then, 4 mL of sodium phenolate and 3 mL of sodium hypochlorite solution were added and the samples shaken well. The absorbance was recorded at 578 nm by a spectrophotometer for 1 h.

(2) Sucrase activity

5 g of air-dried soil samples were weighed into 100mL plastic bottles, and 15mL of 8% sucrose solution, 5mL of phosphate buffer solution (PBS) with pH 5.5 and 5 drops of toluene were added, and the mixture was thoroughly shaken, and then put into a constant temperature incubator for 24h at 37℃. After culturing, the mixture was filtered quickly with qualitative filter paper with a diameter of 11cm (New Star, Hangzhou, China), and 1mL filtrate was put into a 50 mL volumetric flask, with 3 mL 3,5-Dinitrosalicylic acid (DNS) reagent and reacted in a boiling water bath for 5 min, then put under tap water to cool for 3 min. The solution was now orange-yellow, and was diluted it to 50 mL with distilled water. The absorbance at 508 nm was recorded.

(3) Protease activity

2 g of air-dried soil samples were screened by sieving through a 1mm, and placed in 100 mL plastic bottles, and 10 mL 1% gelatin solution prepared with phosphate buffer solution of pH 7.4 and 0.5 mL of toluene were added. Samples were cultured in a constant temperature incubator at 30 ℃ for 24 h. Mixture in the bottles were filtered with a diameter of 11 cm (New Star, Hangzhou, China). 5 mL of filtrate was put into a test tube, with 0.5 mL 0.1 N sulfuric acid and 3 mL of 20% sodium sulfate to precipitate protein, then the solution was filtered into a 50 mL volumetric flask with a diameter of 11 cm (New Star, Hangzhou, China), and 1mL of 2% ninhydrin solution was added. The mixtures were shaken and heated in a boiling water bath for 10min, and the obtained colored solution were diluted with distilled water to the 50 mL on the volumetric flasks. The absorbance at 508 nm was recorded.

(4) Soil nitrate reductase activity

1g samples of fresh soil were weighed into 100 mL plastic bottles, and 20 mg $CaCO_3$ and 1 mL 10 mg/mL of KNO_3 solution were added. After mixing, 1 mL of glucose solution was added to the test samples, and 1 mL of distilled water was added to the control samples to replace glucose. The bottle stoppers were closed and the bottles shaken gently. and placed in a constant temperature incubator at 30 ℃ for 24 h, with the reagent as a blank control. Then, 50 mL deionized water and 1 mL alumite solution were added, left to stand for 20 min, mixed thoroughly and then filtered with a diameter of 11cm (New Star, Hangzhou, China). 20 mL of filtrate was taken into a conical flask, evaporated to dryness in a water bath, and then 2 mL phenol disulfonic acid solution was added for dissolution treatment for 10 min, then 15 mL deionized water was added, and the solution adjusted to light yellow with 10% NaOH added dropwise. Finally, the solution was transferred into a 50 mL volumetric flask and made up to the mark with distilled water, and the absorbance at 508 nm was recorded.

S3. Determination of organic nutrients in *Brassica campestris*

(1) Organic acid

After harvesting, 1 g fresh leaves samples were firstly homogenized with distilled water, and then washed into a 50 mL conical flask. 30mL of water was added and the flasks were incubated in a water bath at 80 ℃ for 30 min. The sample solutions were taken out, cooled and filtered into a 50 ml volumetric flask with a diameter of 11 cm (New Star, Hangzhou, China). The residue was washed with distilled water several times and the wash water added to the volumetric flask, and the solution was made up to the 50 mL mark with distilled water. Then the sample solution was sucked into the 50ml conical flask with a pipette. 3-5 drops of phenolphthalein reagent were added to develop color. 0.1 mol/L NaOH solution was used to titrate to a reddish color, with oscillation of the flask for 30 ~ 60s, and the titration end point was determined as the point at which no further color change occurred, and the consumption volume was record. In order to reduce the test error, each treatment was repeated three times, and the average value of the three titration results was obtained.

(2) Vitamin C

0.3g plant samples were firstly homogenized in an extraction medium containing 5% trichloroacetic acid (TCA), 1mM ascorbic acid (AsA), PBS (pH=7.8), 10% TCA (TCA: water =1:10), 4% 2,2-bipyridine (2,2-bipyridine: water = 4:100) and 3% $FeCl_3$ ($FeCl_3$: water=3:100), and

5 mL 5% TCA solution were added again. The homogenate was transferred into a 10 mL centrifuge tube and centrifuged at 4 ℃ 12,000r/min for 15 min. The centrifuge tube was placed on ice, and the supernatant was carefully removed for analysis. 0.2 mL of the supernatant was added into a reaction system consisting of 1.4 mL PBS, 0.4 mL 10% TCA, 0.4 mL 4% 2,2-bipyridine and 0.2 mL 3% $FeCl_3$. Absorbance at 525 nm was recorded.

(3) Soluble sugar

0.1g fresh plant samples were put into 10mL graduated centrifuge tubes. 4mL of 80% ethanol was added, and tubes were placed in a water bath and oscillated continuously at 80℃ for 30 min followed by centrifugation at 3,000 r/min for 10 min. The supernatant was collected from the centrifuge tubes, added 2 mL of 80% ethanol was added to the centrifuge tubes to extract the residues twice and the supernatants were combined. A little activated carbon was added into the supernatant, and then it was decolorized at 80℃ for 30 min. Deionized water was used to make up to the mark. 1 mL of filtrate and 5 mL of an-throne reagent were mixed to develop a green color, and the absorbance was measured at 625 nm and the concentration was calculated using a standard curve obtained by measuring absorbance of the glucose standard at 625 nm.

(4) Soluble protein

1g of fresh samples were homogenized with phosphate buffer solution. The homogenate was filtered and centrifuged at 13000 r/min for 15 min. 0.1 mL of the supernatant was mixed thoroughly with 4.9 mL Coomassie brilliant blue, G-250. The absorbance at 540 nm was measured and the protein content calculated from a standard curve using bovine serum albumin.

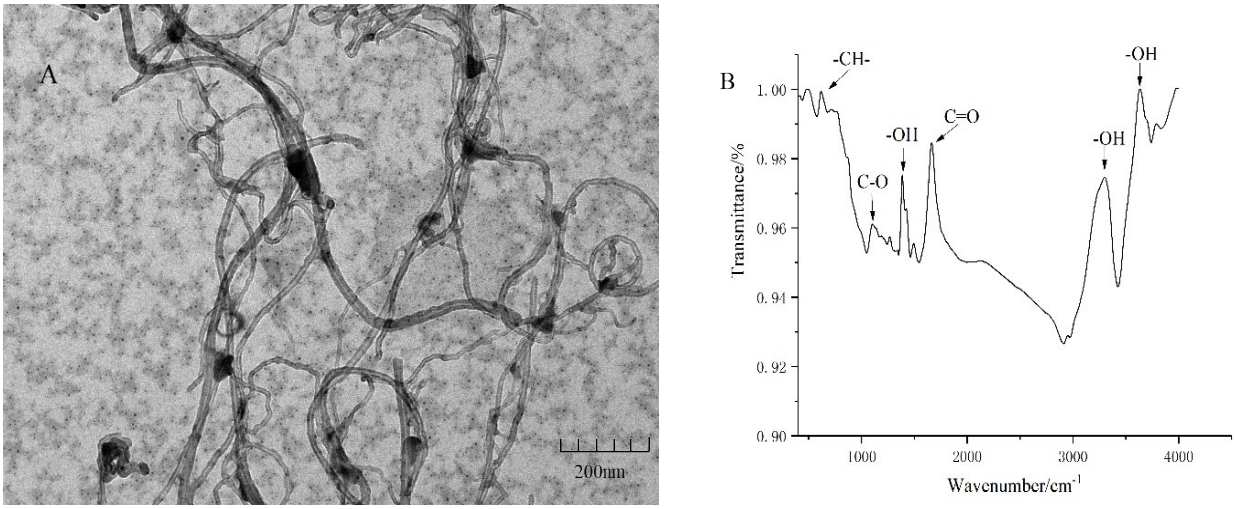

Fig. S1 Transmission electron microscopy images (A) and Fourier transform infrared spectroscopy (B) spectrum of MWCNTs.

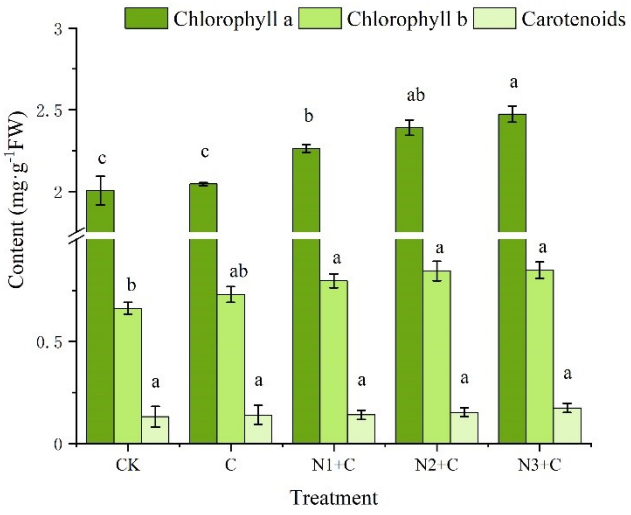

Fig. S2 Chlorophyll and carotenoid content in Brassica campestris as affected by MWCNTs treatment. Different lowercase letters indicate significant difference at $p< 0.05$. CK: conventional fertilization, C: conventional fertilization + MWCNTs, N_1+C: 10% nitrogen reduction +MWCNTs, N_2+C: 20% nitrogen reduction + MWCNTs, and N_3+C: 30% nitrogen reduction + MWCNTs (n=6).

Table S1 Accumulation and proportion of nitrogen from different sources in the whole growth

period of *B. campestris*. Different lowercase letters indicate significant difference at $p< 0.05$ (n=3).

Growth period	Treatment	N_{uptake} mg/kg	$N_{fertilizer}$		N_{soil}	
			Content mg/kg	Ratio/%	Content mg/kg	Ratio/%
15d	CK	32.85±7.38^{a}	9.78±2.46^{b}	29.57±0.86^{d}	23.07±4.91^{a}	70.43±0.86^{a}
	C	43.01±6.27^{a}	18.99±2.58^{ab}	44.22±0.50^{a}	24.02±3.71^{a}	55.78±0.50^{d}
	N_1+C	53.74±1.19^{a}	21.53±1.25^{a}	40.04±1.44^{b}	32.21±0.06^{a}	59.96±1.44^{c}
	N_2+C	42.10±5.77^{a}	16.87±1.89^{ab}	40.20±1.01^{b}	25.24±3.88^{a}	59.80±1.01^{c}
	N_3+C	39.26±11.67^{a}	12.92±3.55^{ab}	33.15±1.81^{c}	26.34±8.12^{a}	66.85±0.81^{b}
30d	CK	66.60±6.02^{c}	33.76±3.13^{c}	50.68±0.12^{a}	32.83±2.89^{c}	49.32±2.89^{b}
	C	82.28±2.63^{b}	40.51±0.84^{bc}	49.29±1.14^{ab}	41.77±2.12^{b}	50.71±2.12a^{b}
	N_1+C	84.39±1.70^{b}	40.19±1.25^{bc}	47.61±0.89^{b}	44.20±1.01^{ab}	52.39±1.01^{a}
	N_2+C	102.33±7.38^{a}	51.42±3.38^{a}	50.30±0.46^{ab}	50.91±4.02^{a}	49.70±4.02^{ab}
	N_3+C	86.67±0.91^{b}	43.40±0.80^{b}	50.08±0.96^{ab}	43.27±1.08^{ab}	49.92±1.08^{ab}
45d	CK	218.58±11.10^{b}	108.01±4.02^{b}	49.54±1.70^{a}	110.56±8.44^{b}	50.46±1.70^{a}
	C	230.84±10.62^{ab}	121.15±6.51^{ab}	52.57±2.69^{a}	109.70±9.44^{b}	47.43±2.69^{a}
	N_1+C	300.07±17.08^{a}	152.82±8.90^{a}	50.93±0.91^{a}	147.25±8.96^{a}	49.07±0.91^{a}
	N_2+C	306.87±31.25^{a}	160.48±17.70^{a}	49.62±1.86^{a}	155.77±20.94^{a}	50.38±1.86^{a}
	N_3+C	192.22±34.81^{b}	94.08±18.99^{b}	48.79±2.84^{a}	98.14±17.03^{ab}	51.21±2.84^{a}