A new approach to distinguishing photosynthetic types of plants. A case study in Northeast China Transect (NECT) platform

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Abstract

Discriminant analysis is an important method in multivariable statistic analysis to show what type an individual should belong to. Based on actual field photosynthetic value set obtained from our research platform, North East China Transect (NECT), a new approach, developed from the concept and principle of discriminant analysis, was proposed to distinguish C_3 and C_4 plants. Indices related to plant photosynthetic capacity measured by an *LCA4* photosynthesis system were selected to build the discriminant model which is based on four related parameters: net photosynthetic rate, transpiration rate, stomatal conductance, and difference in temperature between leaf surface and atmosphere. Compared with other approaches, the present one is fast, straightforward, and efficient.

Additional key words: C_3; C_4; discriminant analysis method; intercellular CO_2 concentration; leaf and atmosphere temperature; net photosynthetic rate; stomatal conductance; transpiration rate; water use efficiency.

Introduction

Plants are divided according to different intermediates of photosynthesis into three types: C_3, C_4, and CAM (Crassulacean Acid Metabolism). Since the discovery of C_4 pathway of photosynthesis and its confirmation in 1965-6, it has been located in at

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least 22 families, 290 genera, and 1700 species in the world (Li 1993a,b). In China 533 species, 41 mutations, and 3 subspecies have been identified (Yin 1997); however, the exact number in this country is not yet clear.

In the past, different photosynthetic pathways were determined chiefly by means of the following three methods (Tang *et al.* 1999): (*1*) On the basis on leaf anatomy, because almost all C_4 plants are of the "Kranz" structure; (*2*) the use of a biochemical index, *viz.*, the ratio of photosynthetic carboxylase activities of phosphoenolpyruvate (PEP) and ribulose-1,5-bisphosphate (RuBP) (Shi 1991); and (*3*) the use of carbon discrimination ratio ($\delta^{13}C$). Different plants have various ratios of $\delta^{13}C$: C_4 species from -8 to -16 ‰, C_3 species from -22 to -35 ‰ (Hattersley 1982, Farquhar 1983, Kalapos *et al.* 1997). Nevertheless, morphologically different leaves of the same species may differ in this parameter (Wang *et al.* 1997). Still another method used was the determination of CO_2 compensation concentration, Γ (Apel *et al.* 1997).

Each of these approaches has its strong and weak points. Some plants were discovered with C_4 metabolic function, but they were short of the "Kranz" structure; hence detecting was difficult to carry out through microscope and physiological indices in many living plants were hard to be measured. Besides, carboxylase activities are easily lost. While stable carbon isotope has often been used to identify different photosynthetic types (Hattersley 1982, Farquhar 1983), this method proved difficult for widespread application in China for the lack of necessary technology.

One of the latest developments arresting public attention made by the IGBP was the study of terrestrial transect as an integral part of its current research on global change (IGBP 1990, Steffen *et al.* 1992, Steffen 1995). NECT (Zhang *et al.* 1997a,b) is a forest-steppe sample with a gradiant of precipitation among the active IGBP-sponsored terrestrial transects in the international arena and it could provide a good research platform (Tang 1999). So we chose NECT here as our research platform to develop a new method for distinguishing plant photosynthetic types. As discriminant analysis is an important method in multivariable statistic analysis to distinguish whatever type an individual should belong to (Pei 1991), we constructed a model on the basis of the C_4 species lists of Li (1993a,b) and Yin (1997). A discriminant standard optimal to some extent required by the method was made according to the C_3 and C_4 groups to minimize deviation, using the four indices of photosynthetic capacity measured by an *LCA4* photosynthesis system.

Materials and methods

Field sampling and measurements: The research platform selected for our research was NECT, one of the four prior key regions in the world, located between 42° and 46° North Latitude, running along the line of 43°30' North Latitude as its central line from east to west, and between the two longitudes of 110 and 132°E, being 1600 km by 300 km.

We investigated NECT in the summer of 1997 together with several ecologists and geographers from the Institute of Botany, Beijing Normal University, Northeast Normal University, Institute of Applied Ecology, Colorado State University, and other institutions. We started in the Hunchun City, Jilin Province, using a Magellan GPS (Global Positioning System) *Field PRO V*™ (California, USA), then positioned one spot every 25 km along 43.5°N eastward, and arrived at the Chunhua Forestry Farm on the borderline between China and Russia. We then turned back along the same latitude westward, arrived at Erenhot in the Inner Mongolian Autonomous Region, the west end of the transect located at the borderline between China and Mongolia.

The number of spots was 81, at each of which we recorded elevation, status of land use, and vegetation types. Meanwhile, we investigated in the region of typical vegetation 30 community types whose large area permits typical sampling for which we measured net photosynthetic rate (P_N) of plants by means of a *LCA4* Portable Photosynthesis System (*Analytical Development Co.*, *ADC,* Hoddesdon, England). The minimal standard leaf area for measuring broad and narrow leaf species was 6.25 and 12.65 cm^2, respectively. If any area measured was smaller or coniferous leaves were used, samples were taken and immediately measured by a *CID-203* Portable Leaf Area Meter (*CID,* Vancouver, Canada). We measured the photosynthesis indices only in clear days, when PAR (photosynthetically active radiation) was from 20 to 2200 μmol m^{-2} s^{-1} between the forest understory and open temperature desert habitats.

Discriminant analysis:

Selection of indices: Six parameters, *i.e.*, net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and the difference in temperature between leaf and atmosphere (ΔT), intercellular CO_2 concentrations (C_i), and water use efficiency (WUE) were selected. The first four parameters reflect the discrepancy in the capacity of carbon assimilation. The last two were selected because the C_4 species have a higher WUE than the C_3 species, and a significant difference exists between C_3 and C_4 plants (in general, C_i of C_3 plants adds up to 70-80 % of the amount in atmosphere, while only 50-60 % in C_4 plants).

Data description: Two-group discriminant analysis shows that original samples belong either to C_3 group from which 51 species were chosen (*i.e.,* 51 cases) or to C_4 group from which 15 species were chosen (15 cases). The two groups should significantly differ only in mean values. As there are only a few C_4 species in an understory, all the species we selected grow in an area of grassland.

Group means and standard deviations between C_3 and C_4 types (Table 1) showed the largest discrepancy in P_N (74.7 %), obviously higher in the C_4 than C_3 group; WUE, E, C_i, g_s, and ΔT followed. The mean differences in C_i were also statistically significant, but with too great standard deviations.

Significance test: We chose two tests, F and Wilks' λ (Table 2). F is the ratio of mean square deviation of within-group to between-group. If F was sufficient large, the between-group mean square deviation was much larger than that within-group, using the significance level of $p<0.05$.

The F-ratio of variable P_N was 34.39 at $p<0.000$, and because of this extremely significant difference the assumption was refused. The difference between C_3 and C_4 functional groups was significant. When we considered a single variable, λ was

Table 1. Group means and standard deviations beween C_3 and C_4 functional types. For abbreviations see the text.

		E	g_s	P_N	C_i	WUE	ΔT	
Means	C_3	6.24	0.92	7.47	268.15	1.43	2.06	51
	C_4	10.54	0.86	29.47	192.30	4.15	2.44	15
	Both groups	7.22	0.91	12.47	250.91	2.05	2.15	66
Standard deviations	C_3	3.53	1.57	4.83	50.23	0.94	1.70	51
	C_4	8.91	0.57	7.97	61.08	2.19	2.18	15
	Both groups	5.47	1.40	10.86	61.40	1.74	1.81	66

a ratio of square sum of within-group to total-group. Low values of λ showed a difference in the between-group (Table 2). The F-ratio of P_N was the largest, and the F-ratios of C_i and WUE were the smallest. Hence only the between-group difference of P_N was significant.

Table 2. Wilks' Lambda (U-statistic) and univariable F-ratios. For abbreviations see the text.

	Wilks' Lambda	Partial Lambda	F (1.59)	p-level
E	0.24	0.937	3.97	0.051
g_s	0.23	0.978	1.33	0.253
P_N	0.36	0.632	34.39	0.000
C_i	0.23	0.998	0.13	0.725
WUE	0.23	0.988	0.72	0.401
ΔT	0.24	0.975	1.53	0.221

Correlation coefficient: In most cases of multivariable analysis, the relationship of variables largely affects the results. For this reason, it was necessary to observe the correlation matrix of selected variables, to average the covariance matrix of all groups, and to calculate a correlation coefficient for the pooled within-group (Table 3). Treating all cases from one sample, we got a total correlation (Table 4): only the coefficients for P_N and WUE reached 0.7, the other ones were small.

Forward stepwise discriminant method: The method by which variables could be selected is called the stepwise discriminant method. In general, there are two ways to input variables: forward and backward (Pei 1991). We selected the forward method chosen in most studies.

The standard norm of this discriminant method is the Bayes function. To enter variables according to their importance, the former variable entered may be rejected because it becomes unimportant with the entrance of later variables; therefore, entering and rejecting in each step had to be statistically tested to ensure that all variables for constructing discriminant function were important.

Table 3. Pooled within-groups correlation. For abbreviations see the text.

	E	g_s	P_N	C_i	WUE	ΔT
E	1.00					
g_s	0.43	1.00				
P_N	0.51	0.07	1.00			
C_i	0.34	0.33	-0.18	1.00		
WUE	-0.48	-0.25	0.35	-0.66	1.00	
ΔT	0.27	0.33	0.32	0.18	0.16	1.00

Table 4. Total correlation. For abbreviations see the text.

	E	g_s	P_N	C_i	WUE	ΔT
E	1.00					
g_s	0.40	1.00				
P_N	0.54	0.02	1.00			
C_i	0.10	0.29	-0.53	1.00		
WUE	-0.12	-0.20	0.70	-0.77	1.00	
ΔT	0.28	0.32	0.24	0.11	0.18	1.00

Results

The final entered variables (Tables 5 and 6) resulted from fourfold stepwise discriminant analysis to six variables and 66 cases of two functional groups of C_3 and C_4 plants. The final variables entering the model were P_N, E, g_s, and ΔT, whereas the variables C_i and WUE were rejected.

Table 5. Summary of discriminant function analysis. Step 4: N of variants in model: 4; grouping: two photosynthetic functional groups. Wilks' Lambda: 0.23232, approx. F (4.61) = 50.391, $p < 0.0000$. For abbreviations see the text.

	Wilks' Lambda	Partial Lambda	F-remove (1.61)	p-level	Tolerance
P_N	0.86	0.27	165.24	0.000	0.65
E	0.25	0.92	5.50	0.022	0.58
ΔT	0.24	0.95	3.24	0.077	0.80
g_s	0.24	0.98	1.13	0.292	0.71
Variables currently not in the model, Df for all F-tests: 1.60					
C_i	0.23	1.00	0.03	0.870	0.67
WUE	0.23	0.99	0.63	0.430	0.26

Discriminant model: The discriminant function was

$$f_g(x) = A_g + \sum_{j \in p} A_{gj}\, x_{j,}, \; (g = 1, 2 \text{ for } C_3 \text{ and } C_4) \quad (1)$$

Table 6. The final variables to enter in the forward stepwise discriminant model. For abbreviations see the text.

Variables	Step	F to entr/rem	df 1	df 2	p-level	No. of vars. in
P_N - (E)	1	174.51	1	64	0.000	1
E - (E)	2	5.72	1	63	0.020	2
ΔT- (E)	3	2.45	1	62	0.122	3
g_s - (E)	4	1.13	1	61	0.292	4

in which $j \in p$ ($p = 4$), and p is the number of variables entering the model, x_j is the entered variable, A_g is prior probability of the g group (here g equals 1, 2).

According to results (Table 7) and Eq. 1, we constructed the discriminant model to distinguish the different plant photosynthetic types as follows:

$$f_{C3}(x) = -1.5493 + 0.1427\,P_N + 0.1035\,E + 0.3768\,\Delta T + 0.1000\,g_s$$
$$f_{C4}(x) = -15.6142 + 1.0542\,P_N - 0.2503\,E - 0.2957\,\Delta T + 0.6491\,g_s$$

Table 7. Discriminant coefficients of C_3 and C_4 plants. For abbreviations see the text.

Group	P_N	E	ΔT	g_s	Constant
C_3	0.1427	0.1035	0.3768	0.1000	-1.5493
C_4	1.0542	-0.2503	-0.2957	0.6491	-15.6142

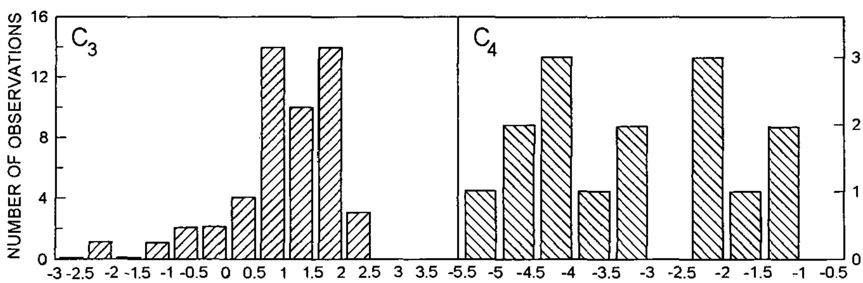

Fig. 1. Histogram for each group. Scores distribution of C_3 and C_4 photosynthetic types.

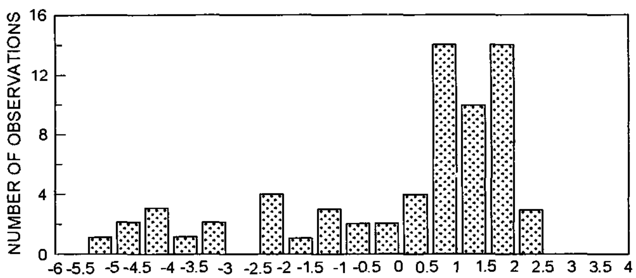

Fig. 2. Two-groups stacked histogram.

Table 8. Returned classification results. Incorrect classifications are marked with *.

	Species	Observed classification	Squared Mahalanobis Distances from group centroids: C_3 groups p=0.77273	C_4 groups p=0.22727	Posterior probabilities: C_3 p=0.77273	C_4 p=0.22727
1*	*Oryza sativa* L.	C_3	10.77	2.81	0.06	0.94
2	*Leymus chinensis* (Trin.) Tzvel.	C_3	1.24	18.75	1.00	0.00
3	*Carex duriuscula* C.A. Meg.	C_3	1.99	21.521	1.00	0.00
4	*Sanguisorba tenuifolia* Fisch. *ex* Link	C_3	1.57	27.25	1.00	0.00
5	*Artemisia frigida* Willd.	C_3	1.50	25.27	1.00	0.00
6	*Melilotoides ruthenica* (L.) Sojak	C_3	0.41	14.36	1.00	0.00
7	*Corispermum* spp. L.	C_3	9.15	34.55	1.00	0.00
8	*Leymus secalinus* (Georgi) Tzvel	C_3	1.84	8.89	0.99	0.01
9	*Puccinellia distans* (L.) Parl.	C_3	3.05	11.33	1.00	0.00
10	*Potentilla chinensis* Ser.	C_3	1.61	12.36	1.00	0.00
11	*L. daurica* var. *shimadae* (Masamune) Masamune *et* Hosokawa	C_3	1.23	13.42	1.00	0.00
12	*Potentilla anserina* L.	C_3	2.74	7.57	0.97	0.03
13	*Sayssurea otophylla* Diels	C_3	6.36	4.19	0.53	0.47
14	*Melilotus officinalis* (L.) Desr.	C_3	3.03	6.59	0.95	0.05
15	*Potentilla bifurca* L.	C_3	1.29	11.76	1.00	0.00
16	*Artemisia annua* L.	C_3	1.43	24.24	1.00	0.00
17	*Astragalus scaberrimus* Bunge	C_3	0.61	16.46	1.00	0.00
18	*Phlomis umbrosa* Turcz.	C_3	2.87	23.64	1.00	0.00
19	*Potentilla fragarioides* L.	C_3	0.54	17.84	1.00	0.00
20	*Artemisia desertorum* Spreng.	C_3	0.17	15.22	1.00	0.00
21	*Astragalus strictus* R.	C_3	6.94	26.90	1.00	0.00
22	*Heteropappus altaicus* (Willd.) Novopokr.	C_3	1.28	16.51	1.00	0.00
23	*Agropyron cristatum* (L.) Gaertn.	C_3	0.36	14.63	1.00	0.00
24	*A. gmelinii.* Web. *ex* Stechm.	C_3	3.28	26.30	1.00	0.00
25	*Veronica incana* L.	C_3	1.51	27.49	1.00	0.00
26	*Potentilla acaulis* L.	C_3	1.30	21.50	1.00	0.00
27	*Ligularia mongolica* (Turcz.) Hand.-Mazz.	C_3	1.38	15.67	1.00	0.00
28	*Grocosmia pottsii* N.E.Br.	C_3	1.20	24.91	1.00	0.00
29	*Filifolium sibiricum* (L.) Kitam.	C_3	0.62	15.83	1.00	0.00
30	*Iris ruthenica* Ker-Gawl.	C_3	0.24	19.25	1.00	0.00
31	*Schizonepeta tenuifolia*	C_3	1.15	14.79	1.00	0.00
32	*Cares idzuroei* Fr. Rt Sav.	C_3	0.37	20.86	1.00	0.00
33	*Scutellaria baicalensis* Georgi	C_3	1.20	26.69	1.00	0.00
34	*Thermopsis lanceolata* R. Br.	C_3	0.57	12.85	1.00	0.00
35	*Aster alpinus* L.	C_3	0.87	19.02	1.00	0.00
36	*Stipa baicalensis* Roshev.	C_3	11.86	27.11	1.00	0.00
37	*Orytropis cachemiriana* Camb.	C_3	15.01	43.63	1.00	0.00
38	*Saposhnikovia divaricata* (Turcz.) Schischk.	C_3	1.22	17.85	1.00	0.00
39	*Allium tenuissimum* L.	C_3	7.45	30.55	1.00	0.00
40	*Asparagus dauricus* Fisch.*ex* Link	C_3	0.83	21.31	1.00	0.00

41	*Echinops gmelinii* Turcz.	C_3	1.49	27.46	1.00	0.00
42	*Ixeris chinensis* (Thunb.) Nakai	C_3	1.40	27.00	1.00	0.00
43	*Caragana microphylla* Lam.	C_3	0.31	16.28	1.00	0.00
44	*C. stenophylla* Pojark	C_3	1.27	19.45	1.00	0.00
45	*Gueldenstaedtia multiflora* (Bunge) Tsui	C_3	1.84	27.14	1.00	0.00
46	*Haplophyllum dauricum* (L.) Juss	C_3	1.99	25.42	1.00	0.00
47	*Stipa breviflora* Griseb.	C_3	1.77	25.77	1.00	0.00
48	*C. intermedia* Kuang *et* H.C. Fu	C_3	1.27	23.35	1.00	0.00
49	*A. gobicus* Ivan. *ex* Grubov.	C_3	1.56	22.13	1.00	0.00
50	*S. gobica* Roshev.	C_3	1.90	24.84	1.00	0.00
51	*S. krylovii* Roshev.	C_3	35.10	49.26	1.00	0.00
52	*Amaranthus retroflexus* L.	C_4	36.51	5.12	0.00	1.00
53	*Beckmannia syzigachne* (Steud.) Fern.	C_4	40.50	6.11	0.00	1.00
54	*Pennisetum flaccidum* Griseb.	C_4	36.95	4.14	0.00	1.00
55	*Chenopodium acuminatum* Willd.	C_4	8.17	2.04	0.14	0.86
56	*Cleistogenes squarosa* (Trin.) Keng	C_4	9.30	1.75	0.07	0.93
57	*Carex enervis* C.A. Mey.	C_4	17.63	1.19	0.00	1.00
58	*Chloris virgata* Swartz	C_4	12.79	1.80	0.01	0.99
59	*Glaux maritima* L.	C_4	58.51	29.38	0.00	1.00
60	*Crypsis aculeata* (L.) Ait.	C_4	42.79	18.26	0.00	1.00
61	*Halerpestes sarmantosa* Liu.	C_4	37.64	10.54	0.00	1.00
62	*Kochia scoparia* var. *sieversiana* (Pall.) Ulbr. *ex* Asche	C_4	11.88	1.51	0.02	0.98
63	*Polygonum senticosum* Franch. *et* Sav.	C_4	28.46	2.99	0.00	1.00
64	*Chenopodium glaucum* L.	C_4	6.44	3.90	0.49	0.51
65	*Chenopodium acuminatum* Willd.	C_4	19.85	0.07	0.00	1.00
66	*Setaria viridis* (L.) Beauv.	C_4	6.15	3.41	0.46	0.54

Scores and returned classification results: Figs. 1 and 2 show the scores for each group and two-groups. We got returned classification results in Table 8 by putting the original values into the model. The methods of testing chosen were squared Mahalanobis distance and Posterior probability.

Only one species (see Table 8), *i.e.*, *Oryza sativa* which should belong to C_3 group, was classified to the C_4 group. Table 9 shows the result of classification matrix. The high degree of accuracy reached at 98.48 % indicated that the species we chose to construct the model were all typical and could represent the different photosynthetic types.

A discriminant rule was made after the acquisition of the model:

$$G(x) = f_{C3}(x) - f_{C4}(x)$$
$$x \in f_{C3},\ G(x) \geq 0$$
$$x \in f_{C4},\ G(x) < 0$$

Now we have a plant denoted by x. With the measurements of P_N, E, g_s, and ΔT, the input of these four parameters to the model resulted in the values of functions $f_{C3}(x)$ and $f_{C4}(x)$. Calculating the value of $G(x)$ by the rule, the plant belongs to the C_3 group if $f_{C3} \geq f_{C4}$, and to the C_4 group if otherwise.

Table 9. Classification matrix.

	%	C_4 functional types	C_3 functional types
C_3	98.04	50	1
C_4	100.00	0	15
Total	98.48	50	16

Discussion

The distinguishing of C_3, C_4, and CAM plants is usually done by determining Kranz leaf anatomy, biochemical index, and $\delta^{13}C$ (Craig 1957, Downton and Tregunna 1968, Downton 1975, Raghavendra and Das 1978a,b, Waller and Lewis 1979, Hesla *et al.* 1982). A discriminant model to distinguish these types based on a set of actual field-measured photosynthetic values has not been built yet. While it is theoretically possible to set up a model using actual parameters, it is difficult to decide which one is more important. Meanwhile, parameters directly related to photosynthetic carbon assimilation pathway should be emphasized. In order to set up a model, we used four indices P_N, E, g_s, and ΔT to detect plant photosynthetic types with a *LCA4* Photosynthesis System and other field photosynthesis measuring instruments.

Firstly, we selected as our research platform NECT, a forest-steppe transect in middle latitude lacking CAM plants. Within the 300 plant species in this survey, only one belongs to CAM, *i.e.*, *Orostachys fimbriatus* (Turcz.) Berge. Thus our model includes only a two-group discriminant analysis without dealing with CAM. It is possible to prepare a three-group discriminant analysis for the area with many CAM plants to improve this model.

Secondly, the same plants at different sites have different capacities for photosynthesis (Table 8). Numbers 55 and 65 are of the same species *Chenopodium acuminatum* Willd., but they grow in different places and differ in photosynthetic capacities. In addition, variations in the time of measurement, climate, *etc.*, should also be considered. For a large area of at least 600 000 km^2, the results measured in this survey were rough due to the limitation of time. Thus, we excluded from our model some indices that show apparent discrepancy in C_3 and C_4 groups, *i.e.*, WUE and C_i.

Compared with other methods, this quantitative model is a new attempt to distinguish different photosynthetic types. Further study of this model would require more values from temperate, tropic, or other zones.

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