Effects of climate factors and vegetation on the CO_2 fluxes and $\delta^{13}C$ from re-established grassland

Yaroslav Bezyk[1,*], *Maxim* Dorodnikov[2], and *Izabela* Sówka[1]

[1]Unit of Ecologistics and Environmental Risk Management, Faculty of Environmental Engineering, Wroclaw University of Science and Technology, Poland
[2]Dept. of Soil Science of Temperate Ecosystems, Georg August University of Gottingen, Gottingen, Germany

Abstract. The relationship between stable carbon isotope composition ($\delta^{13}C$-CO_2) of soil CO_2 flux, vegetation cover and weather conditions was investigated in a short-term campaign at a temperate re-established grassland in Germany. During August-September 2016, we measured surface CO_2 flux with a closed-chamber method at high and low soil moisture content ('wet', 'dry'), with and without above ground vegetation ('planted', 'clear-cut') and estimated the effects of treatments on respective $\delta^{13}C$-CO_2 values. The concentration and stable carbon isotope composition of CO_2 were determined using the gas chromatography and mass spectrometry analyses. The $\delta^{13}C$-CO_2 of the soil fluxes decreased over sampling time for the 'dry-warm' conditions and canopy manipulation. The ecosystem-derived $\delta^{13}C$-CO_2 values (corrected for the atmospheric $\delta^{13}C$-CO_2) which included predominately soil- and rhizosphere respiration were −26.2 ± 0.8‰ for the 'dry-warm' conditions and decreased down to −28.1 ± 1.4‰ over a period of 28 days from late August to the end of September. The decrease coincided with the lowering of CO_2 flux and could be attributed to changes in plant physiological processes at the end of the vegetation season. Though the removal of shoots did not significantly affect the $\delta^{13}C$-CO_2 values as compared with the control, the pattern of further $\delta^{13}C$-CO_2 decrease (down to −28.8 ± 0.8‰) supported the role of living vegetation in a contribution of ^{13}C-enriched CO_2 to the ecosystem respiration.

1 Introduction

Soils as source and sink of the carbon dioxide (CO_2) are an important component in the global carbon (C) balance. The gas exchange between the atmosphere, vegetation and soil is controlled by the complex mechanisms related to various physical (temperature, moisture) and biochemical soil properties (microbial communities and activity, organic matter content), regional geographical features and meteorological conditions (duration of vegetation period, photosynthetically active radiation, precipitation, etc.) [1]. The environmental and biological mechanisms can strongly influence the carbon isotopic

* Corresponding author: jaroslaw.bezyk@pwr.edu.pl

composition of ecosystem-respired CO_2 and play a major role in controlling processes of respiratory isotopic fractionation [2]. In order to predict the response of C balance to environmental changes, it is necessary to determine effects of different climate factors including diurnal and season temperature changes, precipitation level as well as the role of live vegetation (aboveground biomass, plant ground cover) in an ecosystem.

Many studies have examined the main driving factors affecting the soil CO_2 respiration rates, but most of these studies provide contradictory results in relations of soil temperature and soil moisture content. Analysis of field observations in large part demonstrates that the soil temperature is the primary factor determining the rates of soil respiration [3, 4], while other numerous data considers that the effect of temperature is constrained by soil moisture availability [5, 6]. The impact of moisture on soil respiration and microbial activity is more complex than temperature because moisture availability also depends on physical characteristics of soil (texture, porosity, and organic matter content) [7]. Generally, the separating the effect of temperature on respiration from the effect of moisture or vice versa in the field face difficulties, since these parameters tend to vary continuously and inversely.

Grasslands are one of the highly important terrestrial biome types since grasslands hold a large portion of the soil C and serve as C sink. They cover approximately one-quarter of the global land surface, but many of these areas have been turned into managed lands (e.g. farming and agriculture) [8]. Although C sequestration is generally associated with native grassland areas, it was found that a large portion of the CO_2 fixation and C sequestration occurs in re-established grasslands [9, 10]. In such restored grasslands, especially in perennial systems, the microbial community composition could be similar to natural grasslands [11] but the response of such ecosystems to environmental changes, i.e. the sustainability of C balance in them, still remains largely unknown.

Therefore, it is critically important to deepen our knowledge on the mechanisms of C turnover in restored grasslands and the useful technique for this – isotope composition method. The carbon isotopic composition of CO_2 has significant potential as a tool to understand the influence of the environmental changes on carbon transformation mechanisms and predict of the future carbon balance of terrestrial ecosystems. So, the goal of this study was to follow the changes in soil CO_2 fluxes and their $\delta^{13}C$ values in response to the varying climate conditions (temperature and precipitation), and link those changes with the activity of vegetation (via canopy manipulation) in a re-established grassland. We hypothesized that (i) the surface CO_2 flux will be higher under warm weather conditions (‘dry-warm’) as compared to cool rainy weather (‘wet-cool’) due to higher temperature and accelerated soil respiration; (ii) a contribution of plant-derived respiration to the total ecosystem respiration should decrease towards the effects of the treatment on vegetation cover compared to a non-treated plot ('planted', 'clear-cut' conditions), as well as due to end of a vegetation season irrespectively of temperature and moisture regimes.

2 Materials and methods

2.1 Study site

The study site was a re-established grassland on anthropogenic soil located in the Forest Botanical Garden (‘Forstbotanischer Garten’) of Georg-August-University Göttingen in the north-eastern part of the city of Göttingen, Germany (9°57'48.4"E, 51°33'25.2"N) [12]. The dominating plant communities were herbaceous species. The main soil characteristics (0-10 cm) are shown in table 1.

Table 1. The main characteristics of the soil before the beginning of the experiment.

Parameters (units)	Value
Soil bulk density (g/cm^3)	1.12
pH in H_2O	7.58
Organic C (% of dry mass)	4.15
Total N (% of dry mass)	0.28
Soil C:N ratio	15.33
Microbial biomass C (mg C kg^{-1})	980.66
Microbial biomass N (mg N kg^{-1})	114.48

The mean annual temperature at the study site is 8.4°C, the mean annual precipitation is 628 mm, with mild winter and moderately humid summer, and the annual average wind speed is 12 km/h from South-West (SW) [13]. The weather conditions data on the day of the sampling: air temperature, photosynthetic active radiation (PAR) relative humidity, precipitation (all the data are from a meteorological station of the University of Gottingen) [14] are summarised in table 2.

Table 2. Microclimate conditions (temperature and PAR, precipitation) during the treatments, August-September 2016.

Parameters (units)	August 12, 2016 ('wet' conditions)			August 26, 2016 ('dry' conditions)			September 28, 2016 ('planted', 'clear-cut')		
	Min	Max	Aver	Min	Max	Aver	Min	Max	Aver
Air Temp. (°C)	16.2	18.9	17.8	16.2	34.8	27.3	14.3	20.5	18.1
PAR (μmol m^{-2} s^{-1})	-0.02	296.7	113.1	1.5	1482	887.7	-0.01	934	169.3
Precipitation (mm)	0.11	0.11	0.11	0	0	0	0	0	0
Real humidity (%)	88.1	95.3	92.0	47.2	83.2	50.8	65.0	72.0	81.1

The short-term campaign took place in August-September 2016. Despite the descending vegetation season, there were a number of weather events characterized as “hot and dry” (with air temperatures above 28°C and ca. 8 consecutive days of '0' precipitation) and 'wet and cool' (t = +17° C; 0.11 mm precipitation) [14].

2.1 Experimental set-up

For ecosystem CO_2 flux measurements (soil + vegetation), a static chamber sampling system was installed. Chambers (n = 6) consisted of two parts: a ‘cap’ - polypropylene plain tubing of inner diameter 24 cm and 20 cm in height - used to collect the emitted gases; and PVC collar (the lower section) of 24 cm in diameter and 10 cm in height, inserted ~3–4 cm into the soil. The chambers were tightly placed on the PVC collars, to omit leakage from chambers to the atmosphere. The chamber volume was ca. 8 L and covered 0.014 m^2 area. During each measurement, the CO_2 was collected with plastic gas-tight 60-ml syringes equipped with 3-way stopcocks at intervals of 15 min over 45 minutes of chamber exposition time. Such time intervals were chosen to achieve qualitative $\delta^{13}C$ signal in CO_2. Part of gas samples was immediately transferred into pre-evacuated 12-ml glass vials and the rest were kept in 60-ml plastic syringes. All the measurements occurred between 2 and 4 PM time.

The experimental plot had 6 randomly established replicate chambers. Chambers are placed on the soil surface including roots and small vegetation e.g. grass in the chamber area during 1-st and 2-nd treatment groups. The incubation experiments were maintained at the treatment-dependent temperature and moisture content: defined as high precipitation treatment and high soil moisture content (concluded on August 12, 2016) and high ambient

temperature and low moisture content (August 26, 2016) called in this study 'wet', 'dry' conditions. During 3-rd treatment (September 28, 2016), few days before measuring, the soil respiration, all aboveground plant materials inside the chamber collars were cut to the ground to eliminate aboveground plant respiration ('planted', 'clear-cut' conditions). The above-mentioned treatment was further divided into two parts connected to environmental and vegetation factors affecting a total respired CO_2 from a soil surface: 'planted' related to changes in weather (under 'warm') conditions according to the previous treatment periods; and 'clear-cut' as control manipulation for studying of both plant physiology (shoots and root biomass distribution) and surface soil features contributing a variation of soil respiration rates in re-established ecosystems.

The soil temperature and soil water content were also determined at 10 cm in soil depth. The soil chemical properties: total nitrogen and carbon contents, soil acidity (pH), soil microbial biomass carbon (MBC), were determined by collecting soil samples at relevant depth (7–10 cm) adjacent to each PVC collar. The soil moisture content was measured the oven-drying method. The soil pH was measured using the electrode method in volumetric proportions of 1:3 soil:water-extract. The total C/N ratio of soil was determined using the dry combustion method [15]. Microbial biomass C and N were measured by the chloroform fumigation-extraction (CFE) technique [16].

The CO_2 concentrations were analysed on a gas chromatograph (GC 6000 VEGASERIES 2, Carlo Erba Instruments) equipped with Electron Capture Detector (ECD). The stable carbon isotope composition ($\delta^{13}C$) of CO_2 were analysed by the CRDS (Cavity Ring-Down Spectroscope, G2131-i Picarro Inc., Santa Clara, CA). All the analyses were conducted at the Department of Soil Science of Temperate Ecosystems, Georg August University of Gottingen, Germany.

2.2 Calculations and statistical analysis

The CO_2 flux was calculated as a change of concentrations over time using a linear function of the slope derived from a geometric mean regression. Gas concentrations were converted from volumetric into mass units using the Ideal Gas Law considering atmospheric pressure and temperature. For each measurement, the results are presented as means of 3–5 replicates ± standard error. Pearson correlation was employed to examine relationships between the CO_2 and $\delta^{13}C$-CO_2 values, and environmental variables. A one-way ANOVA followed by a Tukey test at $P < 0.05$ was used to identify the significance of differences between the variables [17]. Effects of the ambient temperature, related humidity and their interaction on the total ecosystem respiration were carried out using repeated measures ANOVAs ($P > 0.05$).

A two-end-component mixing model, the 'Keeling plot' method [18] was used to identify the stable carbon isotope composition of the soil CO_2 respiration out of the mixture with atmospheric $\delta^{13}C$-CO_2. As the atmospheric $\delta^{13}C$-CO_2, the value of 8‰ from Atmospheric Concentrations of CO_2 from Mauna Loa, (Hawaii, 2015) was used [19].

3 Results

Compared to the average C:N ratios for the typical grasslands in Germany (value in range 10 to 11) [20], the measured C:N ratio in top soil 10 cm at the experimental plot was substantially higher (15.3, Table 1). Significant increase in C:N ratio in re-established grasslands is associated with an effect of land conversion and illustrates the large capability of soil C storage as compared to N [21].

In general, the total CO_2 flux from a soil surface saturated after about 45 min. The significant positive linear relationships were marked between the CO_2 concentration at the

soil surface and the chamber exposure time (approximately 45 min.): the 'wet-cool' treatment (R^2 = 0.73, $p < 0.05$), the 'dry-warm' treatment (R^2 = 0.99, $p < 0.05$), the 'planted' treatment (R^2 = 0.98, $p < 0.05$), the 'clear-cut' treatment (R^2 = 0.98, $p < 0.05$) (Fig. 1).

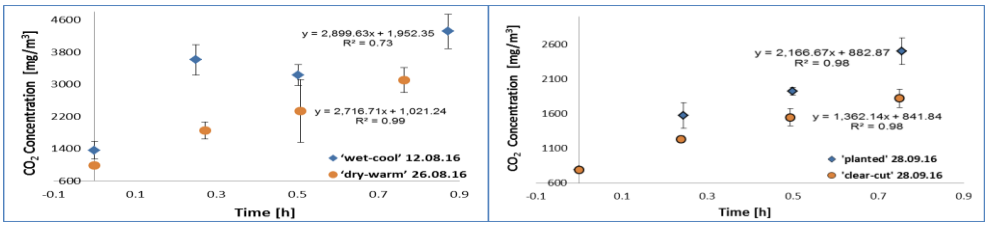

Fig. 1. The mean CO_2 concentration increment during chamber exposure at the re-established grassland. Three dates of measurements and two plant treatments are shown: 12.08.2016 and 26.08.2016 ('wet-cool' and 'dry-warm' weather conditions, respectively); 28.09.2016 (canopy manipulation: 'planted', 'clear-cut'). Error bars show the standard deviation across sub-plots ('wet' 'dry' scenario, n=6; 'planted', 'clear-cut' scenario, n=3). The slope of CO_2 concentration increment over time of a chamber exposition was linearly approximated to estimate the flux.

The ecosystem CO_2 flux did not differ significantly between weather conditions and vegetation manipulations (Fig. 2). Furthermore, the environmental factors tested by ANOWAs (temperature (R^2 = 0.58; F = 2.71, df = 3, p>0.2412) and air humidity (R^2 = 0.79; F = 7.49, df = 3, p > 0.1114)) in a short-term perspective showed positive correlations with total respiration rate, but had no significant effect ($P < 0.05$) on CO_2 flux from ecosystems. Carbon dioxide fluxes during weather condition treatments varied from mean 1640 mg CO_2 m^{-2} h^{-1} ('wet-cool' treatment) to 1555 mg CO_2 m^{-2} h^{-1} ('dry-warm' treatment) and to a minimum 1222 mg CO_2 m^{-2} h^{-1} ('planted' treatment). However, comparing these data at the level of significance ($P < 0.1$) showed a little, but still significant differences between 'wet-cool' and 'warm'/'planted' treatments indicating that flux from ecosystems is depending on changes in ambient temperature. The treatments on vegetation cover 'planted'(soil + vegetation CO_2 respiration), 'clear-cut' '(soil CO_2 respiration) represented the CO_2 flux decreased by 36.9% indicating a significant ($p > 0.0111$) role of vegetation on ecosystem-derived CO_2 flux. There was no obvious effect of temperature changes, tested by ANOWAs (R^2 = 0.74; F = 5.64, df = 3, $p > 0.1407$) on a CO_2 respiration from soil without plant shoots ('clear-cut' treatment).

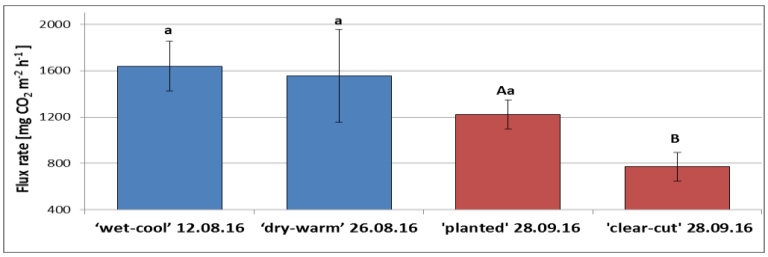

Fig. 2. Mean ecosystem CO_2 flux (soil+rhizosphere respiration) at three dates of measurements: 12.08.2016 and 26.08.2016 ('wet-cool' and 'dry-warm' weather conditions, respectively); 28.09.2016 (canopy manipulation: 'planted', 'clear-cut'). Error bars show the standard deviation of soil CO_2 flux. Significance assessed by one-way ANOVA and Bonferroni test for pairwise comparisons ($P < 0.05$) and denoted in the graph by different case letters. Capital letters indicate which measurements were significantly different and low-case letters are considered non-significant.

There was a positive correlation between CO_2 concentrations and the related $\delta^{13}C$-CO_2 values in different treatments (R^2 = 0.88–0.97, $p < 0.05$) (Fig. 3). The $\delta^{13}C$-CO_2 of the ecosystem and soil ('clear-cut' conditions) respired CO_2 varied in a range from

−11.3 to –23.7‰ and from −10.4 to −23‰ for weather conditions and canopy manipulation, respectively.

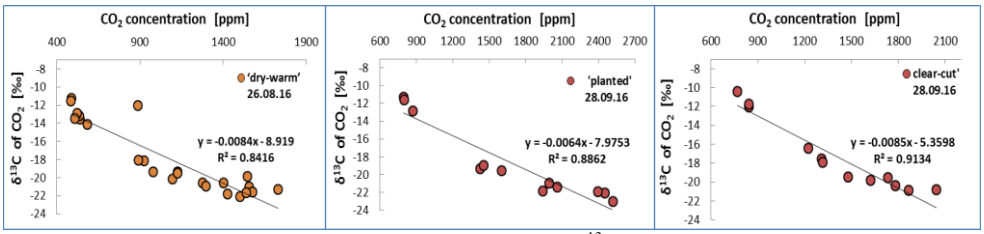

Fig. 3. The relationship between CO_2 concentration and its $\delta^{13}C$ isotopic values of measurement data on 26.08.2016 and 28.09.2016 ('dry-warm' and 'planted') as well as of canopy manipulations ("planted" and 'clear-cut'). There were no data on $\delta^{13}C$-CO_2 available for 'wet-cool' weather conditions.

The mean $\delta^{13}C$ value of the ecosystem (soil + vegetation) CO_2 estimated by Keeling plot method was -26.2‰±0.8 under 'dry-warm' conditions and −28.0‰±1.4 in 'planted' treatment (Fig. 4).

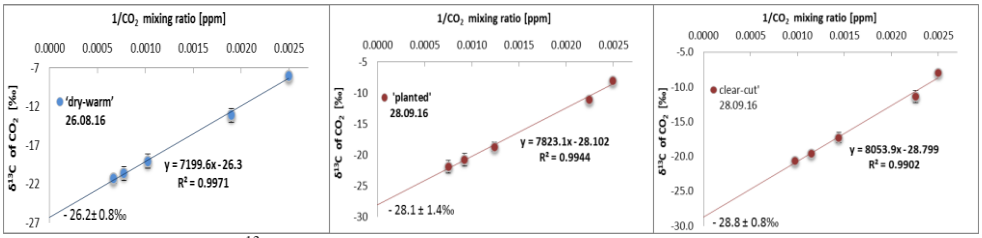

Fig. 4. Keeling plots of $\delta^{13}C$ values and the reciprocal of CO_2 concentrations at measurement data on 26.08.2016 ('dry-warm'); and 28.09.2016, and canopy manipulation: 'planted', 'clear-cut'. Error bars show the standard deviation of the ratio. Data for atmospheric $\delta^{13}C$ (CO_2) value (-8‰) and CO_2 concentration (400.71 ppm) were taken from Atmospheric Concentrations of CO_2 from Mauna Loa, Hawaii, 2015 [19].

The estimated $\delta^{13}C$-CO_2 values of ecosystem respiration in re-established demonstrate the sensitivity of changes in CO_2 ecosystem respiration and its isotopic composition relative to the variation in weather conditions and the role of living vegetation (Fig. 5). It is consistent with the fact that after the treatment on excised shoots ('clear-cut'), the contribution of root respiration was about half (60–65%) of total respiration derived from soils.

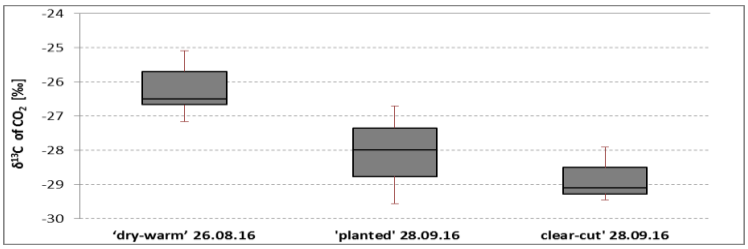

Fig. 5. A box plot of the carbon isotope composition of respired CO_2 at measurement data on 26.08.2016 ('dry-warm' weather conditions); 28.09.2016 (canopy manipulation: 'planted', 'clear-cut'). The box plots represent the spread of 50% (25–75%) of the data range. The horizontal lines indicate the median value. The error bars show upper/lower quartile range (minimum-maximum) of the data values.

4 Discussion

The relationships between ecosystem CO_2 fluxes and environmental factors (weather and vegetation) differ substantially in terms of little, but still, significant differences in temperature and precipitation conditions and high significance of vegetation manipulation conditions. The CO_2 flux from ecosystem was positively correlated with ambient temperature and precipitation and has an average level of significance ($p < 0.1$) between treatments ('wet-cool' and 'warm'/'planted' measurements). The significant interactive effect ($p < 0.05$) between the CO_2 flux and vegetation cover was detected. Cumulative CO_2 flux from ecosystems was highly affected (decreased more than three times) by separating, manipulation on aboveground (removing shoots) versus belowground (roots and microorganisms) respiratory CO_2 sources.

We hypothesized that the surface CO_2 flux will be higher under warm weather conditions ('dry-warm') as compared to cool weather ('wet-cool') due to a higher temperature accelerated soil respiration. Despite the difference was not significant: the CO_2 flux under 'wet-cool' conditions was only by 5% higher than under 'dry-warm' weather. It could be a result of larger soil moisture when water replacing the soil pore space and may increase the CO_2 efflux (physical phenomenon). Increasing was also possible since vegetation respiration was more pronounced under wet conditions. The removal of vegetation under 'warm' conditions decreased the surface CO_2 flux approximately 1.6 times compared with control (with canopy) under the same conditions. The decrease was mainly attributed to the exclusion of shoot respiration and partly due to suppression of root- and rhizosphere respiration.

The difference between the treatments in the $\delta^{13}C\text{-}CO_2$ value was mainly due to factors that affect the isotopic fractionation (biotic and abiotic variables) during of aboveground (biomass, plant ground cover) versus belowground (root + rhizosphere) respiratory CO_2 sources. Indeed, the $\delta^{13}C\text{-}CO_2$ decrease after treatment on vegetation cover ('clear-cut' manipulation), may reflect a decline in the contribution of CO_2 from vegetation respiration to the total ecosystem respiration.

The weather conditions had an impact on carbon isotopic composition of ecosystem-derived CO_2 mainly due to effects of plants physiology in dry and wet periods [3]. The $\delta^{13}C\text{-}CO_2$ values of ecosystem respiration for the 'warm' conditions decreased insignificantly by 2‰ over a period of 28 days (from late August to the end of September). This may be explained by the lowering of photosynthetic activity (partly reflected in CO_2 flux decrease, Fig. 2) at the end of the vegetation season and probably, microbial activity.

Despite there was no significant difference (the decline from 28.1 to 28.8‰) between the $\delta^{13}C\text{-}CO_2$ values in 'planted' and 'clear-cut' treatments, the pattern of further decrease of $\delta^{13}C\text{-}CO_2$ as compared with earlier dates highlighted the role of living vegetation in a contribution of relatively enriched ^{13}C to the entire ecosystem flux. Additional variation in ecosystem respiration and $\delta^{13}C\text{-}CO_2$ flux can be explained by the sum of other environmental conditions such as soil moisture and temperature, as well as by pH and the soil organic matter quality (C:N ratio) in this grassland. Therefore, detailed studies on linking soil organic matter quality, microbial activity and vegetation properties with ecosystem CO_2 fluxes and environmental conditions are necessary for the estimation of current and future C balance.

5 Conclusion

Contrasting weather conditions and vegetation cover, a primary temperature were the main factors controlling ecosystem CO_2 flux in re-established grasslands. However, the differences between treatments in the significance of the relationships between flux rates

and changes in environmental variables were identified. In particular, measured respiration rates were strongly correlated with temperatures and changing water content, but the most significant effects on ecosystem CO_2 fluxes was determined by manipulating vegetation cover. The relationships between carbon isotopic composition of ecosystem-derived $\delta^{13}C\text{-}CO_2$ and environmental conditions showed a minor difference between 'wet' and 'dry' weather treatments as well as slight responsibility for the changing in a contribution of living vegetation. As the processes controlling ecosystem isotope discrimination are more complex and highly unpredictable, the application of continuous monitoring of the $\delta^{13}C\text{-}CO_2$ signal in respiration to predict the response of ecosystems to environmental changes supposed to have the highest potential.

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