

# Revisiting a 37 years abandoned fertilizer experiment on *Nardus* grassland in the Czech Republic

Michal Hejman<sup>a,\*</sup>, Michaela Klaudivová<sup>a</sup>, Jan Štursa<sup>b</sup>, Vilém Pavlů<sup>c</sup>,  
Jürgen Schellberg<sup>d</sup>, Pavla Hejmanová<sup>a</sup>, Josef Hakl<sup>a</sup>,  
Otto Rauch<sup>e</sup>, Stanislav Vacek<sup>a</sup>

<sup>a</sup> Czech University of Agriculture, Kamýcká 129, CZ-165 21 Prague, Czech Republic

<sup>b</sup> Krkonoše National Park Administration, Dobrovského 3, CZ-543 01 Vrchlabí, Czech Republic

<sup>c</sup> Grassland Research Station, Research Institute of Crop Production in Prague, CZ-460 01 Liberec, Czech Republic

<sup>d</sup> Institute of Crop Science and Resource Conservation, University of Bonn, D 53115 Bonn, Germany

<sup>e</sup> Institute of Botany, Academy of Sciences of the Czech Republic, Section of Plant Ecology,  
Dukelská 135, CZ-379 82 Třeboň, Czech Republic

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## Abstract

A set-aside experiment on sub-alpine *Nardo-Caricion rigidae* grassland was identified in the Giant Mts. (Krkonoše, Karkonosze), in which Ca, N and P fertilizers were each applied at three rates on five occasions from 1965 to 1967; since that time plots remained abandoned. In 2004, the effect of all fertilized treatments on sward structure was still visible 37 years after the last fertilizer application. *Avenella flexuosa* and *Anthoxanthum alpinum* were the dominant species in phosphorus fertilized plots whereas *Nardus stricta* was the dominant species in the control as well as in the limed and nitrogen fertilized treatments. The biomass production of *A. alpinum* was higher in all fertilized treatments than in the control but significantly only in one P treatment. The lowest amount of dead aboveground biomass was found in all P fertilized plots followed by two Ca treatments. P, Ca and  $\text{NO}_3^-$  concentrations in the biomass were highest in P treatments and Ca concentration was higher, but non-significantly differing from control, also in two Ca treatments. Soil pH was highest in two Ca treatments and was affected by soil Ca concentration.

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## 1. Introduction

Over the last century, the utilization of mineral fertilizer increased dramatically not only on arable land but even on grasslands in Europe (Elkholm et al., 2005; Kayser and Isselstein, 2005; Salo and Turtola, 2006). This led to a substantial decline in the conservation value of large areas of meadows or pastures and often resulted in the disappearance of endangered plant species from the landscape (Havlová et al., 2004; Lepš, 2004; Marriott et al., 2004). For instance,

in the Park Grass Experiment in southern England started in 1856 (Crawley et al., 2005), species richness was still greatest on plots that experienced no fertilization and lowest on plots where the soil was strongly acidified by the long-term input of ammonium sulphate. Further, in 1941 an experiment combining renovation of original *Nardus*-rich grassland and fertilization was set up on Rengen Grassland Research Station in Germany (Schellberg et al., 1999). In 1990, nearly all the species indicative of extensive grassland management were lost and species richness was lowest in fully fertilized treatments under application of Ca, N, P and K. Decline of species richness as a result of long-term fertilization has been experimentally confirmed from many

\* Corresponding author. Tel.: +420 2 24382129.

E-mail address: [hejman@fle.czu.cz](mailto:hejman@fle.czu.cz) (M. Hejman).

grassland communities in various climatic and soil conditions (Galka et al., 2005; Sammul et al., 2003; Jančovič et al., 1999; Kráľovec and Prach, 1997; Willems et al., 1993).

Forty years after stopping nutrient application in grassland experiment established by Dr. Ludi in the Alps (Ludi, 1959), there was still a visible fertilizer effect on plant species composition, soil pH and concentration of P and N in leaves of selected plant species (Hegg, 1984, 1992; Hegg et al., 1992; Dahler, 1992a,b). This suggests a much more long-term effect of fertilization on the grassland ecosystem functioning than was commonly supposed.

Until now, there has been limited information available about long-term after-effects of fertilization, particularly on *Nardus*-rich swards. To verify the results from Ludi's field experiment, an experiment fertilized from 1965 to 1967 (Štursová, 1974) was resampled in 2004. The aim of this study was therefore to answer the following question: is there a detectable effect of Ca, P, and N fertilization on *Nardus*-rich grassland after 37 years of cessation of fertilization?

## 2. Materials and methods

In 1965, a fertilization experiment to observe the response of *Nardo-Caricion rigidae* grassland to increased nutrient availability was set up in the sub-alpine belt of the Giant (Krkonoše/Karkonosze) Mountains at the borderland between the Czech Republic and Poland (Štursová, 1974). The altitude of the experimental grassland was 1430 m asl, the average annual precipitation within the area was 1380 mm, and the long-term mean annual temperature was 2 °C (Vrbatova Bouda Meteorological Station). The geological substratum was granite underlying low deep humus podzols (Pařalková et al., 2001). The thickness of litter (L), fermentation (F) and humus (H) layers was 5 cm; the mineral Ah horizon was 4 cm thick, dark with 50% of organic matter and characterized by pH (H<sub>2</sub>O) 3.8; the Ae horizon of pure siliceous fine sand was gray and 6 cm

thick, eluviated from silt and clay with pH 3.7. Before primary fertilizer application, the sub-alpine grassland was dominated by *Nardus stricta* followed by *Avenella flexuosa*, *Anthoxanthum alpinum*, and *Carex bigelowii* (Štursová, 1974). Livestock grazing and hay making had occurred there for at least 300 years, but agricultural exploitation had definitely ceased after the Second World War (Hejman et al., 2005a). After setting up the experiment, biomass was collected to investigate the effect of treatments on sward structure only in the initial phase of the experiment and then the plots remained abandoned.

The experiment was arranged in three parallel rows 20 m long, separated from each other by 4 m, on a total area of about 220 m<sup>2</sup>. Each row contained treatments with three fertilizer nutrients applied at three rates (treatment abbreviations: P1, P2 P3, N1, N2, N3, Ca1, Ca2 and Ca3) and one control plot without any fertilization (see Table 1). The sequence of the plots and their fertilizer treatments has been the same in each row, so treatments were not randomly distributed. Further, plots were homogeneous before the first experimental manipulation (Štursová, 1974). A detailed description of treatments applied is given in Table 1. Each treatment plot was 1 m<sup>2</sup> in size, delimited by steel nails in the corners and relocated by metal detector in 2004, and was surrounded by a 1 m buffer zone without fertilization. Fertilizers were applied five times: in autumn 1965, spring 1966, autumn 1966, spring 1967, and in autumn 1967. Ca was also applied together with N and P, and S together with P, as a part of commercial fertilizers (Table 1).

In August 2004 relevés were made in the permanent 1 m<sup>2</sup> plots of all three replicates of each treatment. Cover of each vascular species and the total cover of the herbaceous layer were estimated directly in percentages and nomenclature of vascular plants follows Kubát et al. (2002). Biomass samples were taken thereafter. The sward was clipped to a target height of 3 cm. Compressed sward height was measured by the rising plate meter method originally developed to simply estimate sward height on pastures (see Correll et al., 2003). Cut biomass was weighed and mixed. One-half of each

Table 1  
Description of the investigated treatments

Treatment abbreviation <sup>a</sup>	Nutrient amount applied (kg ha <sup>-1</sup> )				Fertilizer used
	Ca	N	P	S	
P1	60		24	30	CaSO <sub>4</sub> + Ca[H <sub>2</sub> PO <sub>4</sub> ] <sub>2</sub> (20% Ca; 8% P; 10% S)
P2	300		120	150	CaSO <sub>4</sub> + Ca[H <sub>2</sub> PO <sub>4</sub> ] <sub>2</sub> (20% Ca; 8% P; 10% S)
P3	600		240	300	CaSO <sub>4</sub> + Ca[H <sub>2</sub> PO <sub>4</sub> ] <sub>2</sub> (20% Ca; 8% P; 10% S)
N1	20	50			NH <sub>4</sub> NO <sub>3</sub> + CaCO <sub>3</sub> (25% N; 10% Ca)
N2	100	250			NH <sub>4</sub> NO <sub>3</sub> + CaCO <sub>3</sub> (25% N; 10% Ca)
N3	200	500			NH <sub>4</sub> NO <sub>3</sub> + CaCO <sub>3</sub> (25% N; 10% Ca)
Ca1	213				CaO (71% Ca)
Ca2	1065				CaO (71% Ca)
Ca3	2130				CaO (71% Ca)

The total amounts of nutrients applied in years 1965–1967 were five times higher. Doses shown in the table correspond to one application only.

<sup>a</sup> With respect to control.

sample was dried at 85 °C until completely dry and the dry matter standing biomass was calculated. The second half was separated into plant species and dead biomass. Herbage mass of species was determined only for *N. stricta*, *A. flexuosa* and *A. alpinum* due to the uneven and scattered distribution of the remaining sward components. Dry aboveground biomass samples were analyzed for nutrients and minerals concentration. Crude protein (CP), and crude fibre (CF) were determined by the Weende analyses method (AOAC, 1984). Ca, Mg, Na, P, K,  $\text{SiO}_4^-$ , total ash concentration and nitrate were determined by colorimetry, photometry, and by atomic absorption spectrometry in an accredited national laboratory.

Soil cores were taken at four places within each permanent plot after making relevés and clipping biomass samples. Upper litter layers were removed and then the humus Ah horizon was sampled. All four samples from each plot were then mixed, oven-dried at 105 °C, and sieved (<2 mm). All analyses were performed in an accredited national laboratory according to the Mehlich III method to predict plant available Ca, K, Mg and P (Mehlich, 1984). To analyze pH ( $\text{H}_2\text{O}$ ), 5 g of soil was mixed with 25 ml of distilled water.

### 2.1. Data analysis

Treatment effects were evaluated by ANOVA using the STATISTICA 5.0 software (StatSoft, 1995). Multivariate techniques were not used due to the low number of plant species observed. Instead, the cover and biomass data were analyzed separately for individual species present in all plots. *Deschampsia cespitosa*, *Solidago minuta*, and *C. bigelowii* data were not evaluated because of their low frequencies in permanent plots. To determine differences among treatments, the Tukey HSD test was applied after obtaining the significance of ANOVA results.

## 3. Results

There were significant treatment effects on all investigated soil parameters except magnesium concentration (Table 2). Soil pH ( $\text{H}_2\text{O}$ ) was in the range of 3.6–5.2 indicating medium to strong soil acidification. The pH was significantly higher in the treatment Ca3 than the other treatments. The remaining treatments did not differ significantly from the control. Ca concentration in the soil was highest in treatment Ca3, differing significantly from the control and the remaining fertilized treatments. In the field, the accumulation of  $\text{CaCO}_3$  became visible as a scattered white layer in the uppermost humus horizon when soil samples had been taken in this treatment.

There was high variability in the soil P and Mg data. Although concentrations of P generally appeared to be higher in all P treatments, the only significant difference was detected between treatments P1 and Ca3. K concentration

Table 2

Results of the ANOVA analyses on fertilizer effects

Tested variable	F-value	P-value
Soil parameters		
pH/ $\text{H}_2\text{O}$	6.9	<0.001
Calcium	11.3	<0.001
Magnesium	2.4	0.063
Phosphorus	2.5	0.047
Potassium	20	<0.001
Sward characteristics		
Standing biomass	2.5	0.044
Senescent biomass	6.6	<0.001
Sward height	5.4	0.001
<i>Nardus</i> -cover	11	<0.001
<i>Avenella</i> -cover	14	<0.001
<i>Anthoxanthum</i> -cover	3.7	0.007
<i>Nardus</i> -biomass	7.8	<0.001
<i>Avenella</i> -biomass	12	<0.001
<i>Anthoxanthum</i> -biomass	4.1	0.004
Biomass parameters		
Crude protein	2.7	0.03
Crude fiber	3.4	0.01
Calcium	9.4	<0.001
Magnesium	21.1	<0.001
Potassium	27.3	<0.001
Sodium	2.3	0.58
Ash	7.1	<0.001
Nitrate	14.7	<0.001
Silicate	7.2	<0.001
Phosphorus	58.9	<0.001

Degrees of freedom were 9 in all analyses.

was highest in all P treatments, lowest in Ca2 and Ca3 treatments, and intermediate in the control.

*N. stricta* was still reduced in all plots that received P and was replaced by *A. flexuosa* and *A. alpinum* there (Fig. 1). P fertilization treatments showed a stronger effect on the floristic composition than N and Ca treatments (Fig. 1). Plant cover and standing biomass of grass species was in many cases not statistically different from the control, although differences were visible in the plots – higher biomass production of *A. alpinum* than in the control was recorded almost in all fertilized plots. The highest biomass production contributed *N. stricta* in all treatments fertilized with N and Ca (Fig. 1).

Total standing biomass as well as dead biomass and sward height were lowest on plots fertilized with P due to the dominance of soft short growing grasses. In the other plots that were dominated by *N. stricta*, total standing biomass and accumulated litter was higher than in P treatments. In the Ca2 and Ca3 treatments, *N. stricta* differed in its habit from those in the control, but differences were not significant: shorter swards, lower standing biomass and lower dead biomass of all *N. stricta* dominated treatments were recorded there (Fig. 2).

The effect of fertilization on nutrient and mineral concentration in herbage was significant except with Na (Table 2). Herbage P concentration was strongly dependent on the historic P fertilization; highest P concentrations were

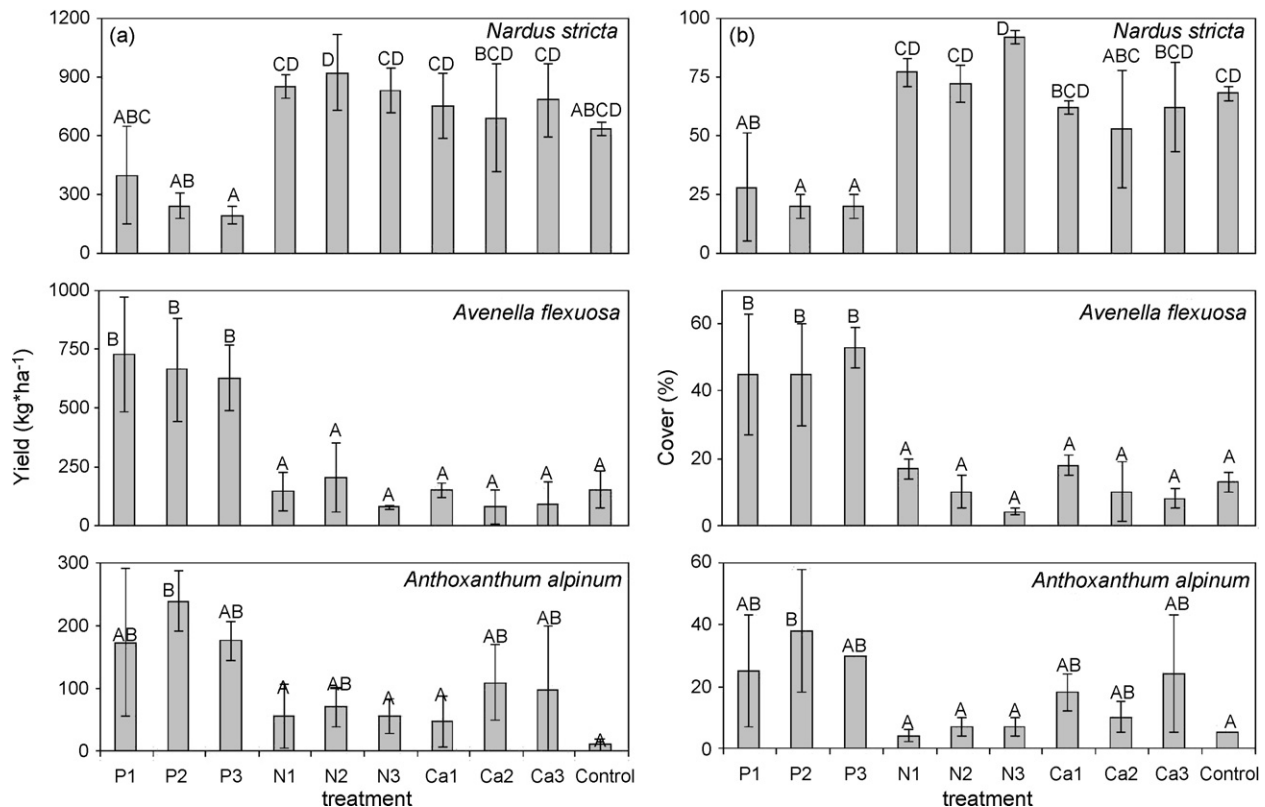


Fig. 1. Standing biomass (a) and percentage cover of dominant species (b) in the investigated treatments. A description of treatment abbreviations is given in Table 1. Values with the same letter within the graph are not significantly different. Error bars represent S.D.

still found in P2, followed by P3 and P1 treatments and was significantly different from the N, Ca, and control plots. The application of P also increased the Mg and K concentration. Na concentration in plant biomass was highest in P1 plots.

The highest total ash concentration was in the control, followed by P1 and P2 treatments. High ash concentration in the control was predetermined by the highest silicate concentration of all treatments. The lowest silicate concentration was in P3 followed by P2, Ca2, and Ca3 treatments. Different plant species composition in P treatments resulted in highest NO<sub>3</sub><sup>-</sup> concentration in plant biomass significantly different from the remaining treatments.

The lowest CP concentrations were found in the unfertilized control, which differed significantly from the P2 and Ca2 treatments. In contrast, the lowest crude fibre concentrations were found in plots that received all doses of P and highest in plots with N fertilization.

#### 4. Discussion

The effects of fertilization, namely P addition, on the floristic composition of the sward were detectable even 37 years after the application. Treatment effects were still visible after P fertilization, mainly due to the dominance of *A. flexuosa* and *A. alpinum* and its eye-catching green color within yellow patches of *N. stricta*. P addition, in contrast to

Ca and N fertilization, strongly affected plant species composition and resulted in a dramatic reduction of the dominant *N. stricta*. However, these effects in the P treated plots cannot be attributed solely to P, as Ca and S had once been applied in the commercial P fertilizer. Hence, the observation of any individual effects of nutrients was not possible. Nonetheless, P concentrations in the aboveground biomass and in the soil were still higher in P treatments, and so the effect of P was probably the most decisive. Finally, the concentration of Ca in biomass was also promoted by P fertilizer application as well probably due to Ca application as a part of P fertilizer, and Ca concentration was higher than in the Ca3 and Ca2 treatments.

The observed strong effects of P on plant species composition was in accordance with results published by van der Hoek et al. (2004), Wassen et al. (2005) or by Bohner (2005). The effect of P fertilization was still highly visible in another experiment that was regularly harvested (Hegg, 1992) rather set-aside as in our study. In the present observations, the calculated removal of P in mown biomass was in the range 1.5–2.4 kg ha<sup>-1</sup> in all treatments, thus P deficiency limited growth of *A. flexuosa* and consequent successional changes towards the original *N. stricta* sward cannot be expected even under 37 years of regular cutting. According to van der Hoek et al. (2004), a persistent P effect on species composition can be ascribed to the fast immobilization and subsequent slow release of fertilizer P



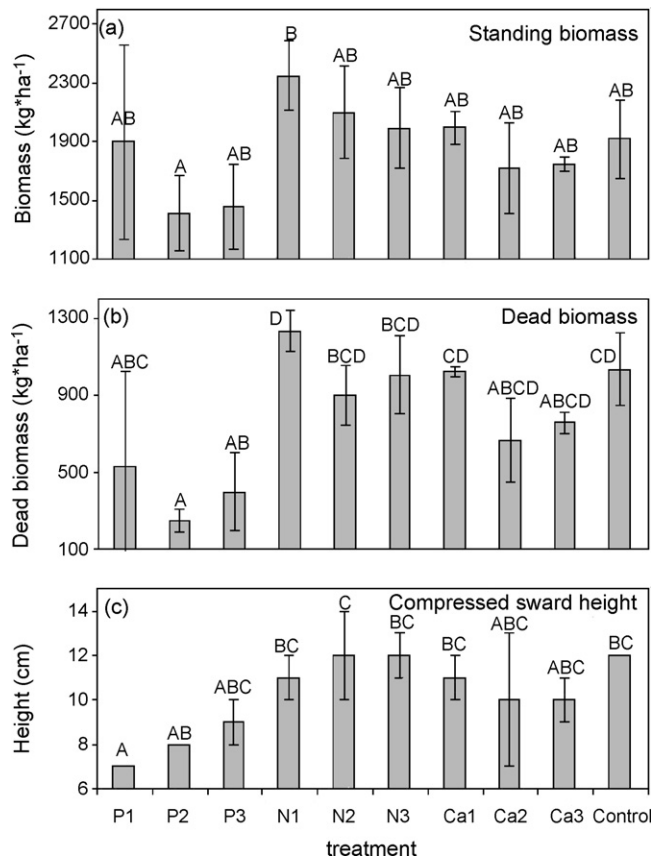


Fig. 2. Total standing biomass (a), dead biomass (b) and compressed sward height (c) in the investigated treatments. A description of treatment abbreviations is given in Table 1. Values with the same letter within graph are not significantly different. Error bars represent S.D.

in peat soils. It was remarkable in the present experiment that P fertilization changed the dominating species composition without any increase in sward height or standing biomass. *N. stricta* in contrast to *A. flexuosa* and *A. alpinum* was probably able to take up P in extremely acid soils and was not hampered by a low P supply, but it lost this advantage in competitiveness when P was supplied. This may be the reason why the concentration of P in the biomass was relatively high in P fertilized plots dominated by *A. flexuosa* and *A. alpinum*. In comparison to these grasses, *N. stricta* was characterized by slow growth rates and biomass decomposition as well as high concentrations of silicate compounds (Hejman et al., 2006) influencing biomass and consequently litter quality which are the main factors affecting the rate of decomposition (Rejmánková, 2001; Gusewell et al., 2002; Aerts et al., 2003).

In contrast to P, the N effect on sward characteristics was visible only slightly after 37 years and there was no significant difference from the unfertilized control. Plots receiving N were dominated by *N. stricta* and their sward structure was, with exception of only non-significantly higher biomass production of *A. alpinum*, similar to the unfertilized surrounding environment of the experiment. In *Nardo-Caricion rigidae* grassland, *A. alpinum* positively

responds to higher nutrient availability, especially to N (Štursová, 1985; Hejman et al., 2005b).

In the case of N fertilization, Ca was also applied as a part of the commercial fertilizer. Therefore, it was not possible to separate the effects of both nutrients in this study. Similar visible long-term effects of fertilization were found 40 years after N application in a *Geo-Nardetum* in the Alps (Dahler, 1992b). Although generally expected, part of the applied N cannot be leached in the short term on extremely poor acid soils under cold conditions. According to Hegg et al. (1992), part of the applied N can relatively quickly be incorporated into microbial (predominately fungal) protein, and therefore leaching could have been delayed. All Ca treatments were dominated by *N. stricta* 37 years after final liming. The effect of liming was as well visible in the field namely in the Ca2 and Ca3 treatments according to higher aboveground biomass production of *A. alpinum* and differing habits of *N. stricta*. Low amounts of senesced biomass indicated probably quicker recycling of nutrients and decomposition of senesced plant material. The color of *N. stricta* swards was darker green than in the control plots. In the Ca3 treatment, the effect of liming was still well visible even in soil reaction and concentration of Ca in the standing biomass predominately composed by *N. stricta*. A complete elimination of *N. stricta* was not detected in the limed plots as commonly recorded in similar studies (Galka et al., 2005) probably because taller grasses were absent at that cold location and thus not able to out-compete stress tolerant species. The impact of liming on *N. stricta* elimination seems therefore to happen indirectly through the support of competitors by fertilization. Ca losses by plant uptake calculated from standing biomass and Ca concentration were relatively small in all treatments ranging from 2 to 3 kg ha<sup>-1</sup> in biomass harvested in 2004. Plant uptake together with recorded white lumps of CaCO<sub>3</sub> in a soil profile of Ca2 and Ca3 treatments led us to the conclusion, that the effect of liming on ecosystem functioning can be a long-term feature even under 37 years of hay making.

The fact that fertilization can be recognized even 37 years after final nutrient application was in accordance with results from an experiment established by Dr. Ludi in the Alps (Hegg et al., 1992). The restoration of extensive grasslands on previously fertilized meadows and pastures can therefore be difficult to accomplish on account of residual nutrients, especially P or Ca, present in the ecosystem for a long time.

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