Structural attributes, tree-ring growth and climate sensitivity of Pinusnigra Arn. at high altitude: Common patterns of a possible treeline shift in the central Apennines (Italy)

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Title: Structural attributes, tree-ring growth and climate sensitivity of *Pinus nigra* Arn. at high altitude: common patterns of a possible treeline shift in the central Apennines (Italy).

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Abstract

European black pine (*Pinus nigra* ssp. *nigra* Arnold) encroachment at increasing elevation has been analysed at four treeline ecotones of the central Apennines (Italy). The study sites are located along a North-South gradient of 170 km across Marche and Abruzzo regions in the Central Italy. The aims of this study were: i) to detect possible common patterns of structural attributes of black pine regeneration at the treeline ecotone; ii) to date the seedlings germination and iii) to assess the climate influence on the pine upward encroachment process also using intra-annual density fluctuations (IADFs) in tree-rings. We sampled 658 encroached black pine trees above the current treeline to the mountain top. All individuals were mapped and their basal stem diameter, total height, annual height increments and other structural attributes measured. One increment core was extracted from stem base of most samples for cambial age determination and detection of intra-annual density fluctuations (IADF). At two sites we also extracted cores at DBH from forest trees to assess climate-growth relationships of black pine. We used multivariate analysis (PCA) to explore the correlation structure of the main tree attributes, regression analysis to relate radial and height increment and dendroclimatic analysis to assess the influence of climate on tree growth and IADF formation.

Most black pine trees were located at high altitude and their structural attributes were similar at the four sites where the pine encroachment process started between 30 and 40 years ago featuring similar germination peaks and growth patterns. Black pine is particularly sensitive to maximum temperatures and IADF occurred in mid-late summer with highest frequency peaks between 2003 and 2004. The pine encroachment process, besides the differences of environmental features and land use histories of the four study sites, appears synchronic and spatially diffused. Consistent tree-growth dynamics and the species adaptation to a warming climate are signals envisaging a possible treeline upward shift.

Keywords

European black pine, Apennines, spatial patterns, tree rings, IADF, climate change.

Introduction

Temperature values have increased globally over the last century and it is considered the main driver controlling treeline formation and dynamics around the world (Körner, 2007). Nonetheless treeline advancement is not a worldwide homogeneous phenomenon and at some sites temperature it is not the dominant limiting factor (Holtmeier and Broll, 2007). Treeline physiognomy seems also to control treeline position and dynamics (Harsch et al. 2009). Harsch and Bader (2011) refer to four treeline primary forms: 1) diffuse, 2) abrupt, 3) island and 4) krummholz and they found that treeline advancement occurred mainly with diffused physiognomy. This form responds better to climate warming, whereas other forms are controlled mainly by dieback and seedling mortality. The influence of temperature can also be masked by interactions with other factors such as precipitation (Daniels and Veblen, 2003; Wang et al., 2006) or cold-induced photo inhibition (Danby and Hik, 2007a). Treeline advancement can also be affected by slope aspect (Karlsson et al., 2007), interspecific interaction (Harsch et al., 2009), physical or geomorphic local conditions (Zhang et al., 2009) and also various anthropogenic disturbances. Treeline locations in relatively undisturbed sites are directly influenced by growing season temperatures and indirectly by altitude, latitude, topography and seed dispersal (Kot et al., 1996). Undisturbed treelines are rare in European mountains, shaped for centuries by human land-use (Dirnböck et al., 2003). In most cases it is very difficult to disentangle the climate from the land-use signal in the assessment of vegetation changes (Gehrig-Fasel et al., 2007). This appears to be the case of the Italian Alps (Motta and Nola, 2001) and even more of the Apennines where severe human pressure and climate change have co-occurred over a very long time span. Treeline ecotones in the Apennines are seldom used as baselines for measuring climate change because of their long history of anthropogenic disturbance. During the Holocene, Apennines treelines were lowered for transforming high altitude forests into grazing areas for herbivores, first wild and later domestic. However livestock pressure has largely decreased over the last 50-60 years and a temperature increase over the last 30-35 years has been recorded in many sites of the region (Brunetti et al., 2006).

Moreover, the typical "abrupt" physiognomy (Harsch et al., 2011) of the local treelines and the life history traits of the dominant tree species, such as *Fagus sylvatica*, have most likely slowed down the expansion process (Stanisci et al., 2005; Pezzi et al., 2008; Gallucci et al., 2010). Other treeline species in the Apennines are *Pinus leucodermis* and *Pinus nigra* subsp. *laricio* diffused in the southern ranges, and *Pinus nigra* subsp. *nigra* that is expanding in the central limestone sites (Piermattei et al., 2012).

Along the Apennines range treeline forms are mainly abrupt and diffuse. European beech (*Fagus sylvatica*) forests tend to form abrupt treeline between 1600-2000 m a.s.l. with very little or no advancement (Stanisci et al., 2005; Pezzi et al., 2008; Gallucci et al., 2010).

Diffuse treelines are less common but more dynamic for the presence of pine forests between 1600 to 2200 m a.s.l.: *Pinus leucodermis* and *Pinus nigra* subsp. *laricio* at the southern sites, *Pinus nigra* subsp. *nigra* at some central sites.

At some central Apennines sites we observed spontaneous pine encroachment above the current treelines mainly formed by European black pine (*Pinus nigra* subsp. *nigra*) plantations for slope erosion control (Piermattei et al., 2012). Black pine regenerates abundantly within its optimal altitude range (500-1600 m a.s.l.), but its scattered presence at higher altitudes (1700-2100 m a.s.l.) seems a recent phenomenon in the Apennines (Richardson, 2000; Piermattei et al., 2012). In the Balkan mountains *P. nigra* is well adapted to extreme xeric sites, steep, rocky slopes and highly erodible soils where growth conditions for other tree species are unsuitable (Poljanšek et al., 2012).

Tree sexual maturity is reached at 15-40 years and the large seed crops are produced at 2-5 year intervals (Isajev et al., 2004). Pollen dispersal and pollination occur from May to June and seed maturation takes place in spring or early summer, about 13 months after pollination. The seeds are completely mature between September and November and they are wind-dispersed when the cones open from December to April of the following growing season (Van Haverbeke, 1990).

The objectives of this study are: (1) to detect possible common spatial patterns of structure and growth attributes of black pine regeneration at the treeline ecotones at four limestone sites of the central Apennines; (2) to date the tree establishment and to check the existence of spatio-temporal patterns along the whole encroachment area; (3) to assess possible relationships of tree-ring growth and intra-annual density fluctuations (IADF) with some climate variables. This study is based on previous works showing that: i) the pine upward shift followed a recent synchronic wave of germination peaks occurred between 1996 and 2000; ii) decreasing livestock grazing as well as climate warming over the last few decades are major drivers of the black pine expansion at high-elevation in the central Apennines (Piermattei et al., 2012).

Materials and methods

Study area

We sampled four treeline ecotones in the central Apennines (Italy) along a 170 km North-South gradient across Marche (Mt. Acuto, ACU and Mt. Vettore, VET) and Abruzzo (Mt. San Franco, SFR and Mt. Sirente, SIR) regions (Fig. 1 and Tab. 1). Meteorological data for climate classification were retrieved from the nearest weather stations for the period 1961–1990 (Tab. 2). Drought are common in July and August and precipitation peaks occur in autumn and early spring. Snowfalls are more common and abundant in late winter (February and March), but snow permanence on the ground is limited and discontinuous.

According to the Rivas-Martinez bioclimatic classification, all sites have a temperate oceanic macrobioclimate; ACU, VET and SIR show a low supra-temperate while SFR an upper supratemperate bioclimate. ACU features a low hyperhumid and VET, SFR and SIR an upper humid ombrotype (Rivas-Martinez and Rivas-Saenz, 2009).

All sites are on calcareous bedrocks but they differ in some physiographic or landscape attributes (e.g. peak and treeline elevation, slope aspect and angle, geomorphology) (Fig.2).

ACU is the Apennine site where we first observed the upward dynamics of the black pine encroachment. It has the lowest elevation but is on a northern slope with an abrupt coppiced beech treeline forest ranging between 1350 and 1450 m a.s.l. The seed bank, is a residual pine plantation located at 300 m a.s.l. down slope.

At VET the peak elevation is the highest; the treeline is abrupt, on a S-SE slope at 1500-1600 m a.s.l., formed by extensive black pine plantations and a few scattered patches of the previous beech forest at the least accessible sites. *Carex humilis* and *Sesleria* spp. dominate the higher elevation limestone grasslands.

At SFR the treeline is also abrupt and formed by an extensive black pine plantation, but on a SW slope. At SIR pine encroachment occurs on a W-SW gentle sloping and heavily pastured karst plateau ranging between 1700 to 2200 m a.s.l. No evident treeline is present nearby and beech forests are at much lower elevation below the grazed area. Even though seeders or residual pine plantations are very scattered and far away from the sampled sites, black pine natural regeneration is dispersed along the entire slope gradient.

At the four sites grazing histories appear different but very difficult to reconstruct due to lack of continuous and reliable data. In the central Apennines grazing season varied locally according to climate and site conditions, but is mainly from May to mid-October and rarely intensive, due to low productivity of these grasslands. Livestock is mainly cows, sheep and more recently horses, but loads decreased up to 50% in the last 40 years due to the increasing abandonment of rural and mountain areas (Pinto-Correia, 1993, Dullinger et al., 2003, Freléchoux et al., 2007).

Cows, sheep and horses are all selective feeders and their preferences are influenced by several foliage attributes (digestibility, shoot biomass, fibre content, nutrient concentrations, level of toxic secondary compounds and spininess) and are highly dependent on what else is available. Pine needles contain terpenes, which make the foliage distasteful and not particularly attractive during the summer when better options are available. Pines can be browsed in winter time when deciduous trees or seedlings are leafless and other vegetation is either unpalatable or covered by snow (Andrews et al., 2000). However no winter grazing is reported for these areas. Goats can cause browsing damage and even death to pine seedlings (Zamora et al., 2001, Torrano and Valderrabano, 2005) but there is no record of goat rearing in the studied areas, at least during the twentieth century. Horses can cause seedlings uprooting or other mechanical damage, but there is no evidence of interference with their germination.

Tree structural attributes

The field data were collected between 2005 and 2012. At ACU, VET and SFR we sampled all the black pine individuals present from the treeline upward. At SIR due to the larger number of trees present across the slope we sampled within a virtual altitudinal transect from 1700 m a.s.l. upward. All sampled areas had a surface comprised between 50 and 120 ha. We mapped all the pines with a Trimble GeoXH GPS device for a total of 658 individuals (72 at ACU, 181 at VET, 254 at SFR and 151 at SIR). For each tree we recorded the following attributes: basal stem diameter, tree height, crown depth, length of stem internodes, needle age (expressed as the number of years of their permanence on the tree branches), tree vigour (according to five classes based on stem and crown damage: 1, no damage; 2, minor damage; 3, medium damage; 4, major damage; 5, dead). To explore the correlation structure of the eight variables (tree structural attributes) and to highlight underlying differences between the four sites, we applied a principal component analysis (PCA) using the PC-ORD 6 statistical package. The statistical significance of the ordination analysis was tested by the Monte Carlo permutation method based on 10000 runs with randomized data.

Tree ring analysis

For the pine trees growing above the treeline a basal increment core was extracted from individuals with basal stem diameter > 4 cm. At VET and SFR, the two sites with a treeline pine forest (Fig. 2), we also extracted two opposite cores at breast height from dominant pine trees selected along the forest edges.

We collected 429 cores from encroaching trees and seedlings (68 ACU, 150 VET, 112 SFR and 99 SIR) and 70 cores from treeline forest trees (20 at VET and 50 at SFR). All cores were mounted on wooden supports and thoroughly polished with progressively finer sandpaper. Tree-ring width measurement, at 0.01 mm accuracy, was provided by the semi-automatic LINTAB system and WinTSAP (Rinntech).

At the four sites the short time series of encroaching pines were visually crossdated. Given their high variability for most individuals we averaged their annual radial increments and compared to their annual height increment by means of regression analysis, in order to assess the influence of other factors.

The ring widths series from the trees at the treeline were visually and statistically checked for measurement errors and crossdated. Each tree-ring series was standardized using the software ARSTAN (Cook, 1985). Since all series are around 20 to 50 years we applied a spline function with a 50% frequency response of 10 years to emphasize higher inter-annual frequency variance (Cook and Peters, 1981). The indexed series were then averaged in the two mean site chronologies and used for the following dendroclimatic analysis.

Climate-growth correlations were calculated using monthly maximum, minimum and mean temperatures and total precipitation data obtained from a 0.5×0.5 degree spatial grid (http://climexp.knmi.nl/) subjected to homogeneity tests and adjustments (Van Oldenborgh, 1999, Van Oldenborgh & Burgers, 2005). The selected climate series, correspond to the closest grid point to the two locations (VET and SRF).

We used DENDROCLIM 2002 (Biondi and Waikul, 2004) with 1000 replications to compute the bootstrapped correlations for the period 1954-2009 at VET and 1966-2009 at SFR. Independent monthly climate variables (T max, T min, T mean and P) were sequenced in a biological year from April of the year prior to growth (t-1) to October of the year of growth (t).

On the tree-ring series of encroaching pines, we recorded presence and frequency of intra-annual density fluctuations (IADF). These are considered tree-ring anomalies or false rings (Wimmer, 2002) and appear after a significant alteration of cambial activity due to withdrawal of normal radial growth, featuring either production of latewood-type cells in the earlywood or earlywood-type cells in the latewood (Fritts, 1976). IADF are mainly climate driven and can be useful indicators of tree adaptation to changing environmental conditions (Vieira et al., 2009, De Luis et al., 2007, 2011).

The annual frequency of IADF in the tree ring series (F), was first calculated with the ratio (Osborn et al., 1997):

$$F=N/n$$

where N is the number of trees where IADF were present in a given year, and n is the number of observed trees. Since changing the samples depth can generate a bias in the variance of the frequency series an adjusted IADF frequency has been computed as follows:

$$f = Fn^{0.5}$$

where f is the stabilized IADF frequency (Osborn et al., 1997).

We assessed the influence of climate on the tree ring series IADF frequency with a nonparametric Spearman correlation analysis. We used mean annual temperature and total precipitation values from the gridded climate data, which have been used to calculate some climatic and bioclimatic indices as the Aridity Index (AI – De Martonne, 1926), the Rain Factor (RF – Lang et al., 1976) the annual Ombrothermic Index (OI), the Ombrothermic Index of the two warmest months (OI2), and the Ombrothermic Index of the warmest quarter (OI3), (Rivas-Martinez et al., 1999).

Results

Tree structural attributes

Tree size of encroached individuals is highly variable at the four study sites: the diameter at stem base ranged from 0.5 to 55 cm (mean 9 cm, std.dev 7.5); the tree height ranged from 0.12 to 8.4 m (mean value 1.4 m and std.dev 1.3). The smallest trees in diameter and height were recorded at

SFR, the largest ones at SIR (Tab. 3). Mean age was very similar at all sites and varied between 12 to 19 years. Most pine trees were located between 1700 and 1800 m a.s.l. and the highest elevation (2155 m a.s.l.) was reached at SIR. At ACU treeline and mountain peak altitudes are lower and therefore most trees grow between 1500 and 1600 m a.s.l. However no correlation was found between age and altitude at the 4 sites (Fig. 3).

The planted pines growing at the treeline margin have different size and age. The mean diameter and mean height at VET and SFR are respectively 38.5 cm (SD ± 7.7) and 10.8 m (SD ± 6.1) and 28.5 cm (SD ±1.3) and 10.2 m (SD ± 2.5).

The multivariate ordination analysis (PCA) on tree structural attributes revealed a high within-site variability (Fig. 4). However the short distance in the ordinal environment between the centroids of the four sites suggested minor differences among sites, confirmed also by the high overlay of their convex hulls. SIR and VET are very similar due to the higher share of larger and older saplings, whereas SFR displayed a different pattern for the larger percent of younger trees. The needle age (Nee) appeared positively related to tree vigour, but both these variables were not correlated to tree size. Globally the first two principal components were significant ($p < 0.001$, Monte Carlo test) and accounted for a cumulative 67.08% of the total variation (Table 4). PC1 described variations of tree size (diameter and height) and age, whereas PC2 expressed a gradient of tree health or vigour.

Growth patterns

The cambial age frequency distribution revealed that pine recruitment started slightly earlier (around 1974) at SFR, SIR and VET than at ACU (in 1981). In all sites, the cambial age frequency approximated a normal distribution (Shapiro-Wilk $W > 0.5$), typical of even-aged processes, with maximum peaks between 1992 and 2002. A later period (1998-2003) of maximum recruitment was recorded at SFR (Fig. 5). The positive and significant correlation obtained by regression analysis of radial and height mean increments suggested the presence of a common growing pattern at all sites

(Fig. 6). ACU and VET regression lines have steeper slopes. ACU shows also the most fitted linear pattern (r = 0.74).

We used respectively 38 and 20 series to build site chronologies of planted pines growing at the treeline margin at SFR and VET. The two time series have similar trends and are well synchronized. Mean cambial age at DBH is 41 yrs (SD ± 9.73) at VET and 36 yrs (SD ± 9.98) at SFR. This large within-site variability may be largely attributed to different planting phases.

Climate influence on tree growth

Pinus nigra responses to climate at the two treeline forests are variable and somehow controversial (Fig. 7). The only common response at both sites is the negative and significant correlation with May precipitation. At SFR alone June precipitation has a positive influence on tree-ring growth. At VET maximum temperatures have a positive effect in May (t) and a negative one in July (t-1). At SFR pine is globally more sensitive to temperatures, in fact maximum, mean and minimum ones all influenced tree-ring growth. Positive correlation was found for both maximum temperature in March of the current year (t) and April of the previous year (t-1).

All IADF detected in encroached trees are earlywood-type cells in the latewood band near the ring closing border (Fig. 8), revealing that cambial activity first decreased or stopped during early summer and then recovered during late summer and early autumn. IADF frequency distributions are not globally homogenous: at ACU and VET values are higher and the curves have approximately normal shapes; at SFR values are much lower and distributed along the entire time span; at SIR the distribution pattern is irregular showing an increasing trend in the last years. Nonetheless maximum peaks are synchronic, occurring in 2003 at VET and SFR, and in 2004 at ACU and SIR (Fig. 9).

The correlation analysis between IADF annual frequency and climatic variables showed similar pattern especially for ACU and SIR; at SFR all values obtained are not statistically significant. Mean annual temperature is significantly and positively associated ($\rho > 0.4$, $p < 0.05$) at all the sites except for SFR (Table 5). Annual precipitation is negatively and significantly correlated only at

ACU and SIR. The climatic and bioclimatic indices (OI, OI2, AI and RF) were negatively and significantly correlated to IADF frequency at ACU, VET and SIR sites.

Discussion

In the central Apennines *Pinus nigra* encroachment of abandoned pastures is a widespread and continuous process at mid-slope elevation (up to 1500 m a.s.l.), enhanced by the nearby abundant seed sources provided by nearby pine plantations. Less common is the irregular advancement of black pine trees above the current treeline, as observed at the four study sites. Here most trees are growing between 1700 and 1800 m a.s.l. whereas some have unexpectedly trespassed the threshold of 2000 m a.s.l. (2090 m at VET and 2155 m at SIR) (Fig.3).

Structural attributes at the four sites showed a high *within-site* and a low *between-site* variability. The former can be related to the large extension of the sampled areas due to the scattered distribution of the pine individuals, and to the numerous limiting growth factors at high altitude, such as extreme climate conditions, irregular topography, shallow soil, rock fall, debris flow and land use changes (Holtmeier and Broll, 2005). In these conditions the presence of safer sites enhanced growth performance and also cone production (Piermattei et al., 2012). At SIR, given the more favourable topographic conditions of a moderate sloping karst plateau, trees have a larger mean size. Furthermore geomorphic related disturbances are less frequent here compared to the other sites, all with steeper slopes. This was confirmed by the significant lower percentage of severely damaged (by rock or debris fall) trees recorded at SIR.

Nonetheless the between-site variability is very low, suggesting that the structural and tree-growth variables are globally very similar (Fig. 4). In this study the tree vitality was not correlated to size and age of trees, therefore we may assume that their vigour loss or mortality are mainly controlled by external factors.

Site features and grazing histories are different at the four treeline ecotones, therefore the strong overall similarity exhibited by the PCA supports the influence of a major common driver to the encroachment process. Since the same hypothesis was posted in a preliminary study concerning only two sites (ACU and VET) (Piermattei et al., 2012) we believe that the similar outcomes obtained in a wider study area provide further evidence of an overall climate control on the pine upward shift.

This hypothesis is also supported by the synchronic pattern of the pine pioneering encroachment above the treeline initiated 30-40 years ago at all sites. Tree-ring dating confirmed that pine germinations begun not later than 1974 at three sites except ACU, where started not later than 1981. Cambial age frequency distribution curves have similar trends with modal peaks between 1992 and 2003 decreasing in the following years, especially at ACU. At VET and SFR the presence of extensive pine plantations provided a larger seed supply and a more abundant dissemination. At ACU and SFR seed was provided by fewer and distant parental trees at lower elevation, strengthening the role of wind as a dispersal vector and a crucial driver to guarantee the pine pioneering process. The seeds of black pine are very light and wind-scattered by secondary dispersion, a step-wise process that favours seed transport at long distance and higher altitude (Johnson and Fryer, 1992; Greene and Johnson, 1997).

At the treeline the most limiting factor to height growth is the low temperature (Körner, 2007), reducing the meristematic activity regardless of the photoassimilate abundance (Rossi et al., 2007). In our sites, despite their different altitudes and microsite conditions, the correlation between mean radial and height increments is positive and significant suggesting that tree growth is consistent in both directions, scarcely affected by other external factors and possibly controlled by climate.

Summer drought is very likely to affect radial growth, not only in Mediterranean species (Cherubini et al., 2003). In xeric sites of Austria the growth of *Pinus nigra* is mainly controlled by spring-summer moisture availability (Strumia et al., 1997; Leal et al., 2007). In drought sensitive areas of the Mediterranean basin, European black pine tree-ring growth is mainly influenced both by summer precipitation and temperature (Fernandez et al., 1996, Lebourgeois, 2000), but contrasting effects with lack of correlation are also reported in south-western Spain (Martin-Benito et al., 2008).

The sensitivity to temperature and precipitation of planted pines at VET and SFR is variable and somehow controversial (Fig. 7). The negative correlation of May precipitation (often snow at that altitudes) at both sites could be related to their direct effect in shortening the growing season and possibly reducing the amount of incoming solar radiation and of the photoassimilate produced. The positive effect of June precipitation at SFR can be explained with its more southern location and the warmer aspect (SW) of the slope.

The positive responses to spring maximum temperatures (March at SFR and May at VET) can suggest their crucial role at the beginning of the growing season, but with delayed effects according to local climate differences, warmer and more xeric at SFR than VET.

In the central Apennines the length of the growing season can change yearly, starting from April to June and ending between October and November. A warmer start can reactivate earlier the cambium prolonging the period of earlywood production (Gricar et al., 2006; Rossi et al., 2008) before summer drought conditions could occur. This effect appears more pronounced at SFR both during the year of tree-ring formation [March (t)] and prior to it [April (t-1)]. The negative correlation of July (t-1) Tmax at VET is likely related to the indirect and lagged effect of high temperature on increasing evapotranspiration. The tree-ring formation is limited more by the previous year July Tmax than by the current ones, which is reasonable in a xero-thermic treeline location where wood formation can withdraw during the warmest and driest month.

The IADFs and other anatomical features can also be used to assess the climate influence on tree growth patterns (Novak et al., 2013). Several studies showed a relationship between IADF formation and climate (Wimmer et al., 2000; Rigling et al., 2001; Campelo et al., 2006; De Micco et al., 2007; Novak et al., 2013).

In our samples the IADFs are mainly located within the latewood portion of the ring (Vieira et., al 2009, De Luis et al., 2011, Novak et al., 2013) indicating the occurrence of resumed favorable conditions after a summer stress which reactivated cambial activity toward the end of growing season (De Luis et al., 2011; Camarero et al., 2010; Novak et al., 2013).

The IADFs frequency in all sites, except SFR, are somehow correlated to climatic conditions, positively with mean annual temperatures and negatively with annual precipitation. Increasing temperature can cause a higher evapotranspiration, induce water stress and altering cambial activity by anticipating the production of latewood cells. Increasing precipitation, especially in summer, allows trees to avoid summer dormancy and to conclude normally the seasonal growth as confirmed by the OI2 and OI3, that are indexes calibrated on precipitation of the two warmest months and of the warmest quarter.

Maximum peaks of IADFs frequency at VET and SFR occurred in 2003, the warmest year of the last century (Beniston, 2004) and in 2004 at ACU and SIR. We did not find any significant correlation between IADFs frequency and elevation but with tree size and radial increment. Other studies found that the presence of IADFs is higher in younger trees (Vieira et al., 2009; Novak et al. 2013) and in trees with wider rings (Villalba and Veblen, 1994; Rigling et al., 2001; Copenheaver et al., 2006; De Luis et al., 2007). It has been also proved that younger trees have usually a longer growing season (Rossi et al., 2008) and that they respond faster to changing environmental conditions (Villalba and Veblen, 1994).

Conclusions

The encroachment of *Pinus nigra* above the current treeline is a recent non-linear natural process observed in the central Apennines, that we measured at four representative sites. This secondary succession, which induced pine seedling establishment at unexpected high elevations, appears controlled by at least three different co-occurring factors: i) the presence of local seed sources of European black pine, a highly pioneering species, ii) the decreased livestock grazing pressure and possibly, iii) the climate warming trend. Pine seeds were provided by near and also distant parental trees in extensive or residual plantations. Suitable colonizing space was made available with the declining grazing pressure throughout the last 40-50 years, especially where herbaceous species were not too competitive. Finally the climate warming, recorded in the area, seemed to have favoured the upward expansion of pine seedlings (Piermattei et al., 2012).

The *Pinus nigra* encroachment appears in general as a successional wave featuring synchronic major peaks between 1995and 2000, and a general decrease in the following years. The overall similarity of the tree structural and growth attributes at all sites is counterbalanced by a high within-site variability. Tree-ring growth and IADF frequency seem to confirm the suitability of the species to a changing environment. The results also suggest the overall influence of a climatic driver to the black pine upward shifting process. The research will continue on additional sites and with more detailed geostatistical and tree-ring analyses for assessing the ascending dynamics of the treeline ecotones and the specific contribution of climate change.

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Tables

Table 1. Main physiographic characteristics at the four study sites.

	Acuto (ACU)	Vettore (VET)	San Franco (SFR)	Sirente (SIR)
Latitude	43° 27' N	42° 81' N	42° 45' N	42° 15' N
Longitude	12° 42' E	13° 26' E	13° 38' E	13° 60' E
Peak elevation (m a.s.l.)	1668	2476	2132	2348
Slope aspect	N - NW	S - SE	W - SW	W - SW
Treeline elevation (m a.s.l.)	1350-1450	1600	1450-1600	-
Treeline forest type	Beech coppiced forest	Black pine plantation	Black pine plantation	-

Table 2. Meteorological data (mean annual temperature and annual precipitation) for the period 1961-1990 obtained from the local weather stations nearest to the four study sites.

Sites	Meteorological station (name, altitude, coordinates)	Mean Annual Temperature (°C)	Annual Precipitation (mm)
ACU	Fonte Avellana (689 m a.s.l.) 43°28′N–12°40′E	11.6	1210
VET	Montemonaco (987 m a.s.l.) 42°53′N–13° 19′E	11.1	1708
SFR	Campotosto (1430 m a.s.l.) 42°33'N, 13°22'E	7.8	994
SIR	Aquila (685 m a.s.l.) 42°22'N-13°21'E	11.7	732

Table 3. Summary statistics of main tree structural attributes at the four study sites. M = mean; SD = standard deviation; D = diameter at stem base; H = tree height; H_In = mean internode length; Age = cambial age at stem base.

Sites	Trees (n)	D (cm)		H (cm)		H_In (cm)		Age (yrs)	
		M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
ACU	60	6.8	2.5	100.2	40.9	6.8	1.8	12	3.2
VET	147	6.1	3.7	100.8	61.8	8.5	3.1	11	5.4
SFR	192	4.7	3.6	72.7	54.1	6.9	2.7	10	3.3
SIR	69	7.7	4.8	114.9	67.0	8.0	2.4	12	4.5
Total	*468*	*5.9*	*3.9*	*91.3*	*59.4*	*7.5*	*2.8*	*11*	*4.3*

Table 4. Principal component loadings for the first two principal components at the four sites. Loadings greater than 0.4 are indicated in bold.

	Axis	
	PC1	PC2
% of variance	*46.68*	*20.40*
Cum. % of variance	*46.68*	*67.08*
p	*0.0001*	*0.0001*
Stem diameter (**D**)	**0.488**	-0.091
Tree height (**H**)	**0.492**	-0.163
Cambial age (**Age**)	**0.405**	0.207
Age by internodes (**Age_I**)	**0.463**	0.080
Needle age (**Nee**)	-0.076	**-0.596**
Vigour (**Vig**)	-0.118	**-0.596**
Mean internode distance (**H_In**)	0.350	-0.326
Crown shape (**Cro**)	-0.005	-0.315

Table 5. Correlation coefficients (Spearman's ρ) between IADF annual frequency and climatic variables at the four study sites. T = mean annual temperature, P = annual precipitation, OI = Ombrothermic Index, OI2 = Ombrothermic Index of the two warmest months, OI3 = Ombrothermic Index of the warmest quarter, RF = Rain Factor, AI = Aridity Index. Significant values are indicated in bold character (* = $p<0.05$; ** = $p<0.01$) .

Site	T	P	OI	OI2	OI3	RF	AI
ACU	**0.603****	**-0.557****	**-0.618****	**-0.448****	**-0.507***	**-0.609****	**-0.588****
VET	**0.459****	-0.327	**-0.415***	**-0.447****	-0.329	**-0.358***	**-0.358***
SFR	0.167	-0.205	-0.218	-0.036	-0.056	-0.212	-0.208
SIR	**0.492***	**-0.433****	**-0.472****	**-0.502****	**-0.570****	**-0.465****	**-0.440****

Figure captions

Figure 1. Location of the four study areas in the Marche (ACU, VET) and Abruzzo (SFR, SIR) regions, in the Central Italy.

Figure 2. Landscape view of the treeline ecotones at the four study sites of Central Apennines: a) Mt. Acuto (ACU); b) Mt. Vettore (VET); c) Mt. San Franco (SFR); d) Mt. Sirente (SIR).

Figure 3. Scatter plot and regression lines of elevation and age of pine trees at the four treeline sites.

Figure 4. Principal component analysis of structural attributes surveyed at the four treeline ecotones. Full line arrows represent tree descriptors: D = diameter at root collar; H = tree height; H_In = mean height of internodes; Age = cambial age; Age_In = estimated age by number of internodes; Cro = crown shape; Vig = tree vigour; Nee: years of permanence of needles on the branch stem. Grey symbols indicate sampled trees at each site (triangles: ACU; circles: SFR; squares: VET; diamonds: SIR), black symbols are the centroids of all the trees at the same site. Grey polylines are convex hulls indicating the maximum surface area occupied by trees belonging to the same site. The first and second principal component were significant ($p < 0.001$, Monte Carlo test) and accounted respectively for 46.7% and 20.4% of the total amount of variation.

Figure 5. Frequency distribution (in %) of sampled individuals according to their cambial age. Age was determined from increment cores extracted at the lowest possible height at the stem base of pine trees having diameter ≥ 4 cm (68 cores extracted at ACU, 150 at VET, 112 at SFR and 99 at SIR).

Figure 6. Scatter plot of mean radial and mean height increments at four study sites (black circles: ACU; white squares: VET; grey triangles: SFR; white circles: SIR). The R^2 values are respectively 0.55, 0.52, 0.31, 0.26, all significant at $p < 0.05$.

Figure 7. Correlation functions between mean tree-ring indexed chronologies and total monthly precipitation and mean monthly maximum temperatures for the previous (small letters) and current (capital letters) growth year. Standardized coefficients were obtained by dividing the mean correlations by their standard deviations after the bootstrap replications. They express the significance of monthly parameters. Black horizontal lines are the $p < 0.05$ significance thresholds (Student t ±1.96).

Figure 8. Intra-Annual Density Fluctuations (IADF) within the latewood of encroached *Pinus nigra* in two different samples and in two different years: A) IADF type L with earlywood-like cells within the latewood; B) IADF type L^+ with earlywood-like cells between latewood and earlywood of the following tree ring (Campelo et al., 2013).

Figure 9. Yearly distributions of stabilized IADF frequencies at the four study sites.

Fig.1

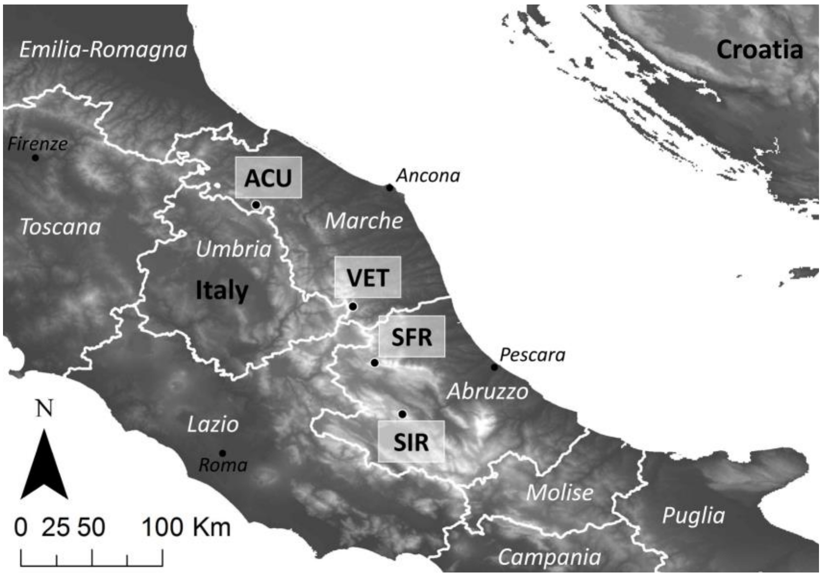

Emilia-Romagna
Croatia
Firenze
ACU
Ancona
Toscana
Marche
Umbria
Italy
VET
SFR
Pescara
Abruzzo
N
Lazio
SIR
Roma
Molise
0 25 50 100 Km
Puglia
Campania

Fig.2

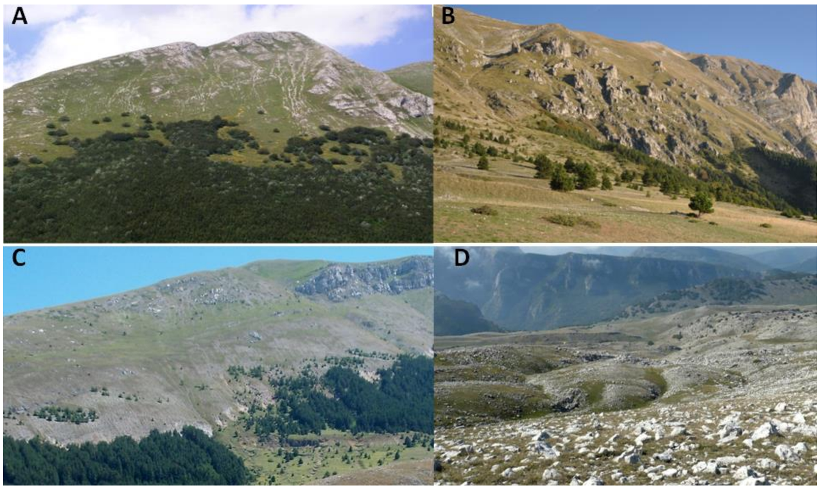
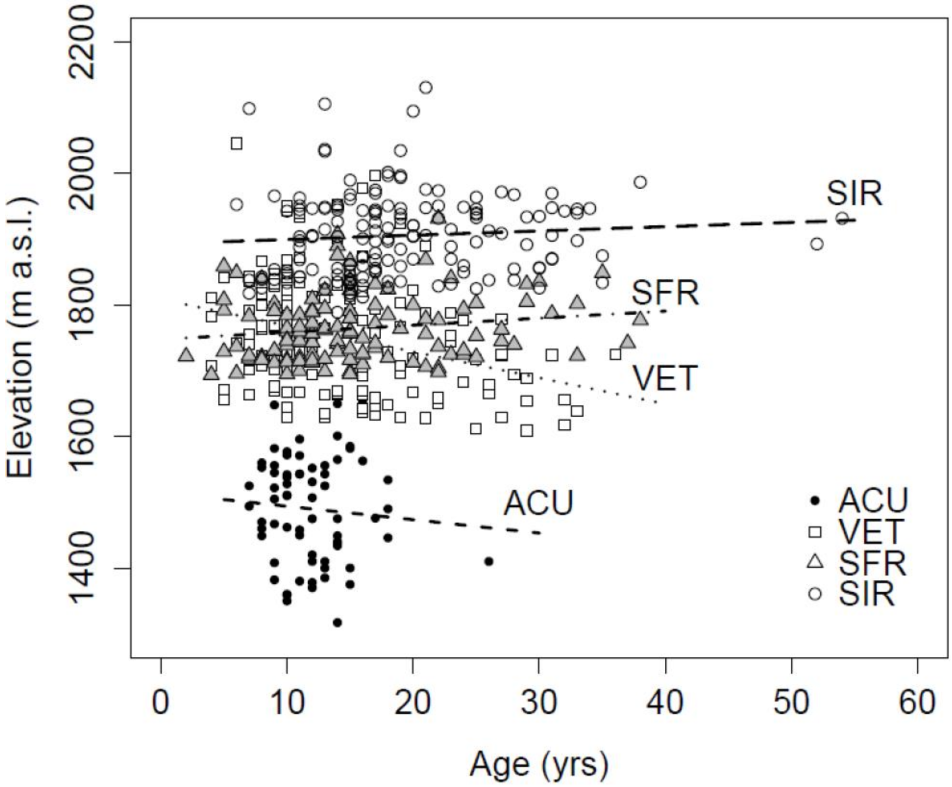
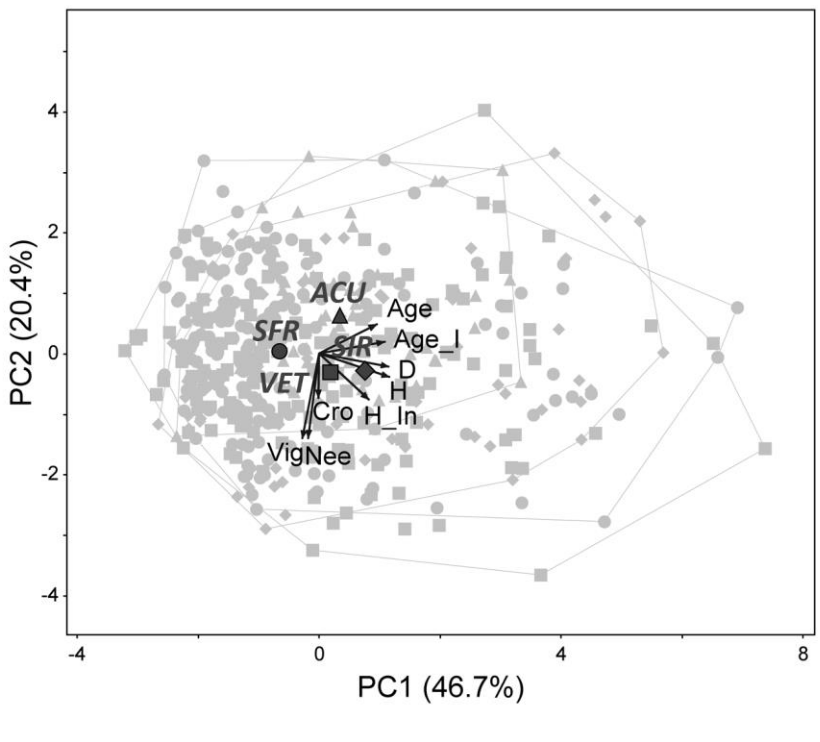

A
B
C
D

Fig.3



Elevation (m a.s.l.)
Age (yrs)
SIR
SFR
VET
ACU
ACU
VET
SFR
SIR

Fig.4



PC2 (20.4%)
PC1 (46.7%)
ACU
SFR
SIR
VET
Age
Age_I
D
H
H_In
Cro
VigNee

Fig.5

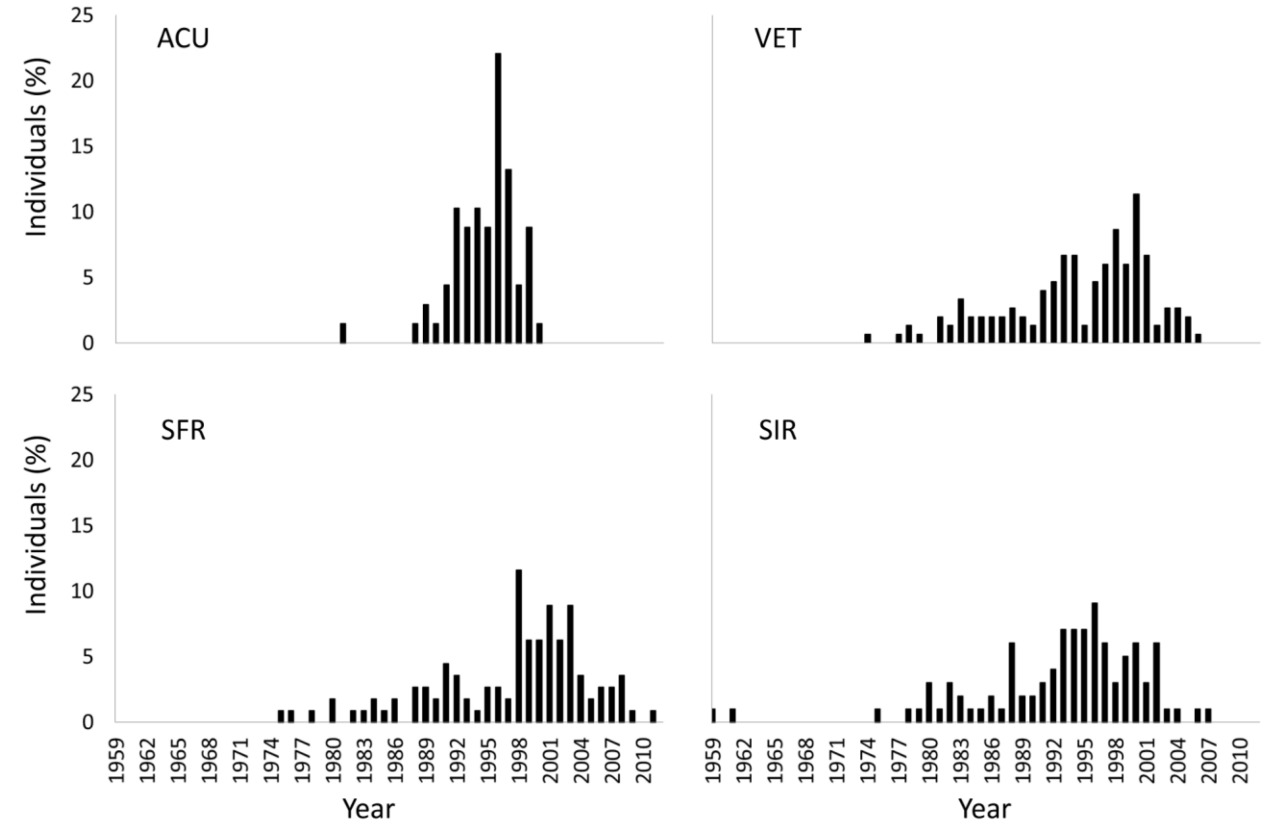

ACU
VET
SFR
SIR
Individuals (%)
Year
0
5
10
15
20
25
1959 1962 1965 1968 1971 1974 1977 1980 1983 1986 1989 1992 1995 1998 2001 2004 2007 2010

Fig.6

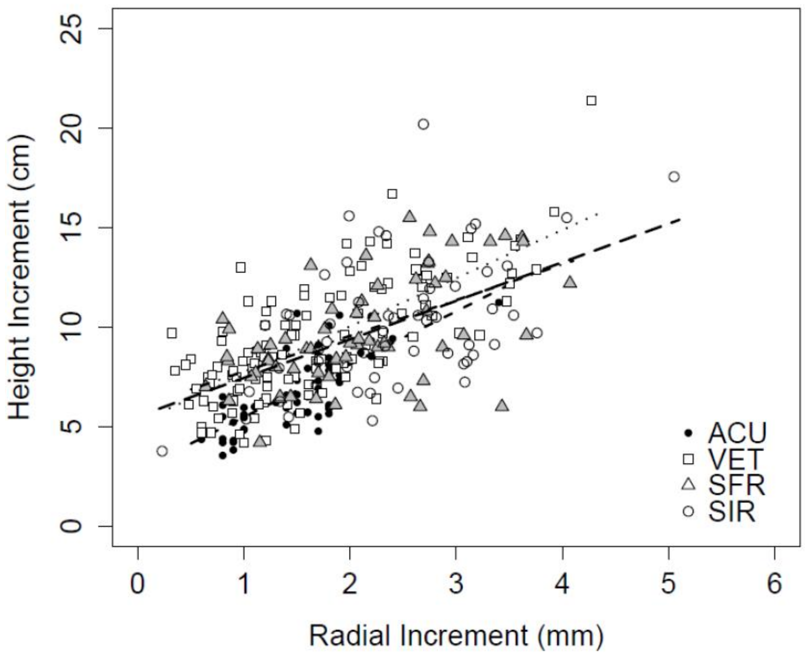

Height Increment (cm)
Radial Increment (mm)
ACU
VET
SFR
SIR
0
5
10
15
20
25
0 1 2 3 4 5 6

Fig.7

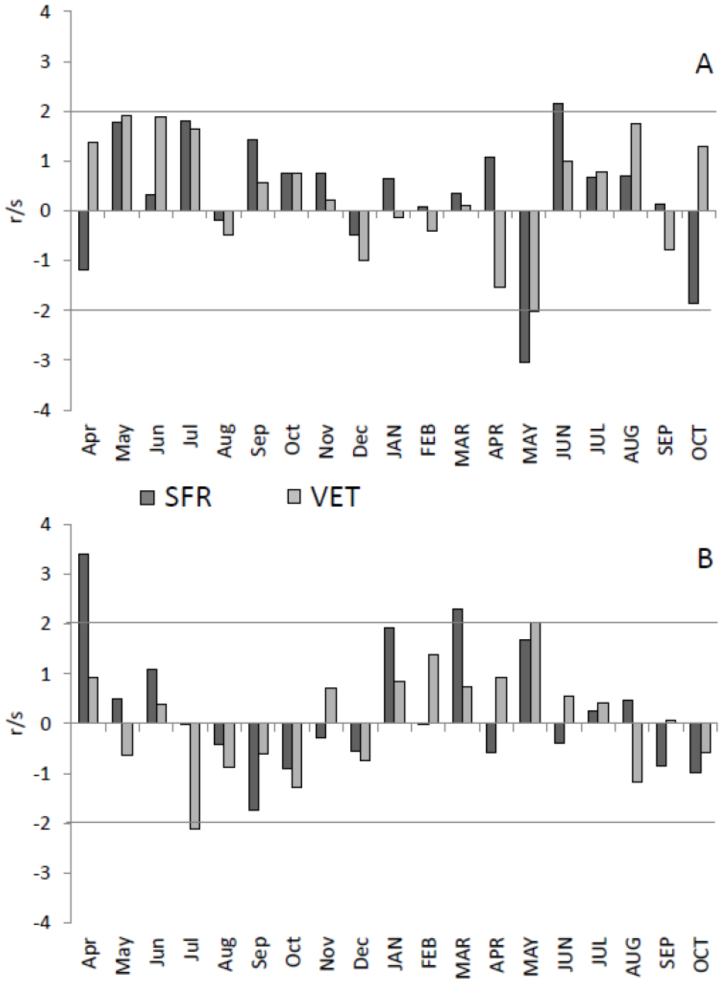

A
B
r/s
SFR
VET
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT

Fig.8

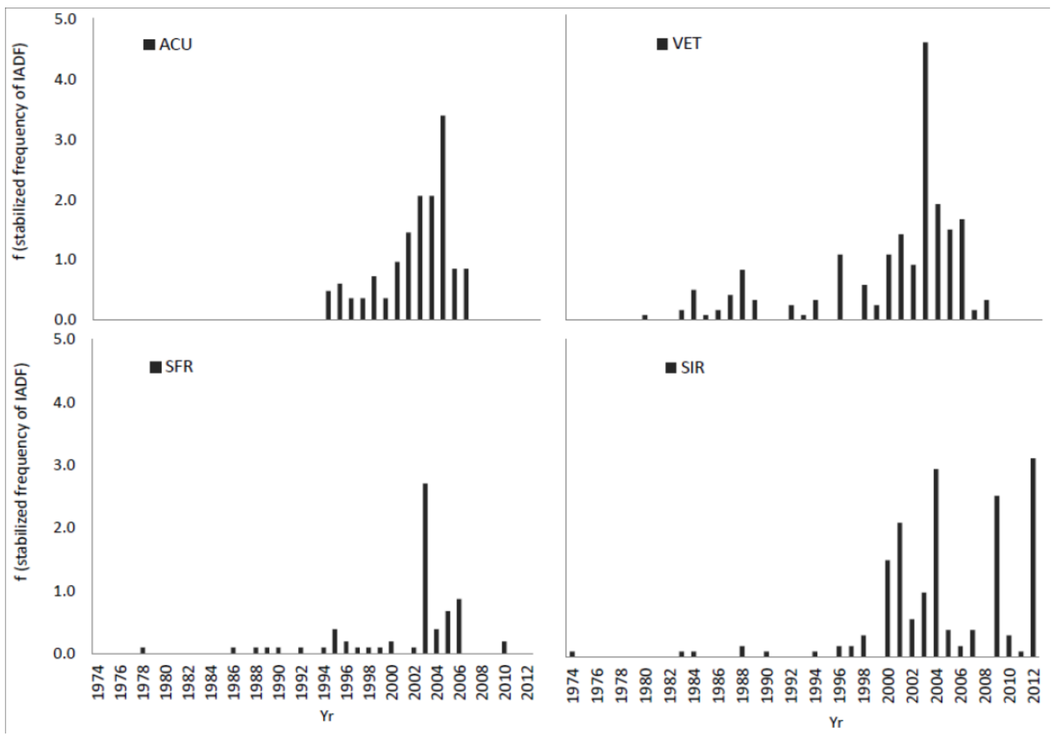

ACU
VET
SFR
SIR
f (stabilized frequency of IADF)
Yr

Fig.9