Relationship between severity of trunk decay of *Pinus koraiensis* and soil properties around roots

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Abstract The decay rate of standing Korean pine (*Pinus koraiensis*) in natural forests can be as high as 50% and is likely influenced by the soil properties and nutrient and water status of the site. To clarify the relationship between the severity of tree decay and soil properties in order to prevent decay in a natural mixed forest in the Xiaoxing'an Mountains, wood strength of standing trees was nondestructively assessed, and the severity of decay of extracted wood cores was quantified based on differences in mass between two decayed increment cores extracted at breast height and an intact increment core near the decayed ones. Soil samples from the critical root zone (non-rhizosphere) of each tree were analyzed for chemical properties and microbial composition. The abundance of chemical elements (especially total N and K) and the species richness of soil microbes increased as decay severity increased. Fungal number (FN) and actinomycetes number (AN) were related to decay severity ($R^2=0.504$). Bacterial number (BN) was higher than FN or AN, but had a minor effect on tree decay. Path analysis showed BN might indirectly inhibit decay by affecting FN.

Decay severity was not significantly correlated with either soil fungal or bacterial diversity. These results suggest that forest managers need to monitor levels of fungi and total N and total K levels to reduce the decay of Korean pine.

Keywords *Pinus koraiensis* · Soil chemical properties · Soil microbial properties · Trunk decay degree

Introduction

Korean pine (*Pinus koraiensis* Siebold & Zucc.), one of the dominant tree species in natural forests of the Xiaoxing'an Mountains in North China, is irreplaceable for providing structural stability to local forest ecosystems. Its wood is prized for its high quality and strength and easy processability (Lv and Qu 2010). However, wood decay of *Pinus koraiensis* in natural forests has become a serious problem (Li et al. 2014; Wang et al. 2015). Tree decay is an essential biological process that produces carbon dioxide and water with a release of energy to maintain forest processes (Bednarz et al. 2013), but wood quality is diminished by wood rot fungi during the decay process (Shortle and Dudzik 2012). Such decay affects tree health and timber production by degrading wood cell walls and reducing wood mechanical and physical properties. Factors such as forest density, site conditions (e.g., elevation, slope, humidity, temperature, light, and soil), hydrology and human and animal activities (Chen 1959) need to be assessed for their contributions to tree decay and loss so that appropriate management strategies can be designed.

Among the site conditions, soil properties are critical to the availability and absorption of water and nutrients by trees and affect the rhizosphere of standing trees and thus influence tree decay, as shown by our previous studies on

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soil physicochemical properties in relation to tree decay (Sun et al. 2015). We found that enhanced C/N ratios and pH in the soil of the root zone was associated with more decay in Korean pine trunks. Hietala et al. (2016) noted that the incidence of decay in Norway spruce also increased as the pH increased from 3.8 to 6.4. These studies indicate a close relationship between tree decay and soil chemical properties.

These properties in turn are closely related to soil microbial communities (Hu et al. 2006; Martiny et al. 2006; Pietri and Brookes 2008); the abundance and composition of the communities can influence soil chemical properties, which can then affect chemical composition (Cai and Huang 2016). In addition, soil microbes can infect roots which could eventually cause trunk decay. However, little information is available on direct relationships between tree decay and soil microbial properties and how soil microbial quantity and diversity contribute to the incidence and severity of decay. Such information is critical to control tree decay; when the relationships are clearer, management practices can be designed to adjust soil properties to improve forest soil and reduce decay in Korean pine.

Therefore, the objective of this study was to analyze the relationship between the degree of tree decay and soil microbial properties based on our previous research and the effects of soil chemical and microbial properties on decay severity in standing trees of *Pinus koraiensis*. This basic data will lead to the development of forest management practices that reduce decay in this important species.

Materials and methods

Study area

The study area in northeastern China is located in the Liangshui National Nature Reserve on the southern slope of the Dailing region, the largest branch of the Xiaoxing'an Mountains. It has a cold continental monsoon climate with an annual average temperature of 1.4 °C. Field tests and surveys of *Pinus koraiensis* were carried out in a 30-ha sample plot in the 18th block of the Liangshui Forest District. The main soil type is dark brown forest soil; the dominant tree species is *Pinus koraiensis* accompanied by species such as *Ulmus pumila* Trautv., *Abies fabri*, *Betula costata* Trautv., and *Tilia tuan* Szyszyl.

Determination of tree decay severity

In the 18th block, the sample plot was divided into 750 quadrats, each 20 m × 20 m, and 100 quadrats containing *P. koraiensis* were then selected along an S-shaped transect in the plot. For ensuring the selected tree types were distributed over a wider area, no more than three trees were tested in

each quadrat. A total of 200 trees were visually observed for decay symptoms and the internal physical structure of the wood tested using 2D stress wave tomography (Arbotom, Rinntech, Heidelberg, Germany) and a Resistograph (model 4453, Rinntech) according to the manufacturer's instructions. Based on the nondestructive test results, nine trees with decay and one healthy *P. koraiensis* trees were chosen as sample trees, each in a different quadrat. For each sample tree, two decaying increment cores were extracted at breast height, and an intact core extracted near the decaying ones. Each wood core was oven-dried (105 °C) to a constant mass and weighed. The mass of the decayed wood cores (m_1) and intact wood cores (m_2) were used to calculate the loss in mass of wood cores (S) as $S = [(m_2 - m_1)/m_2] \times 100$ (Liu et al. 2015) to describe the severity of tree decay.

Soil chemical properties measurement

Soil samples of 500 g were collected from four sampling sites in the critical root zone (rhizosphere) of the selected trees. Surface vegetation, branches and leaves were removed before drilling with an auger to obtain samples from the A horizon at 6–20 cm depth. The 44 soil samples were placed in a sterile bag, which was sealed and taken immediately to the laboratory. Half of the samples were used for chemical analyses, and the other half for microbe isolations and stored at –20 °C until used.

For chemical analyses, soil samples were air-dried, screened with a 2 mm mesh, and fine roots removed. Total and hydrolyzed N, total and available P, total and available K contents were determined according to forest industry standards in China (LY). Total N was measured using the Kjeldahl method (LY/T 1228-1999). Total K was tested using acid dissolution-flame photometry (LY/T 1234-1999). Total P was measured using anti-colorimetric determination of acid-soluble molybdenum and antimony (LY/T 1232-1999). Hydrolyzed N was measured using alkali hydrolysis diffusion (LY/T 1231-1999). Available K was extracted using ammonium acetate-flame photometry (LY/T 1236-1999). Available P was tested using the anti-colorimetric method and molybdenum and antimony with sodium hydroxide extraction (LY/T 1233-1999).

Soil microbial isolation and quantification

From each fresh soil sample, 1 g was diluted to 10^{-2} – 10^{-7} with distilled water and shaken 15–20 min. Each dish of medium received 0.05 mL of the 10^{-5} to 10^{-7} dilutions to plate bacteria on beef extract peptone, 10^{-3} to 10^{-5} for fungi on Martin's medium and 10^{-2} to 10^{-4} for actinomycetes on Gaoshi No. 1 medium (Table 1; Xu et al. 2019). Each concentration was plated out on three dishes.

Table 1 Composition of media to isolate bacteria, fungi and actinomycetes from soil

Type	Composition	Mass (g)	Volume (mL)
Martin's medium for fungi	KH ₂ PO ₄	1.0	
	Glucose	10.0	
	Agar powder	15.0	
	MgSO ₄ ·7H ₂ O	0.5	
	Peptone	5.0	
	1/3000 Bengal red solution		100
	distilled water		900
Beef extract peptone medium for bacteria	Beef extract	3.0	
	Peptone	10.0	
	Agar	15.0	
	NaCl	5.0	
	Distilled water		1000
Gaoshi no. 1 medium for actinomycetes	Soluble starch	20.0	
	KNO ₃	1.0	
	K ₂ HPO ₄ ·3H ₂ O	0.5	
	Agar	15	
	MgSO ₄ ·7H ₂ O	0.5	
	NaCl	0.5	
	FeSO ₄ ·7H ₂ O	0.01	
	Distilled water		1000

The plates were inverted and incubated at 37 °C. Colonies were counted using an automatic colony counter (Interscience scan300) after two days for bacteria, three days for fungi and seven days for actinomycetes. The number of colonies per gram of fresh soil was then calculated.

Fungal and bacterial diversity estimation

DNA was extracted from soil microorganisms for PCR amplification of the internal transcribed spacer (ITS) sequence to estimate fungal diversity and the 16S rDNA sequence to estimate bacterial diversity.

Extraction of DNA

The Fast DNA Spin Kit for Soil Kit (MP Biomedicals Biomedical Co., Irvine, CA, USA) was used to extract total DNA from 500 mg in a Lysing Matrix E tube with 978 µl sodium phosphate buffer and 122 µl MT buffer. The tube was placed in the Fastprep instrument and rotated at 6.0 m s⁻¹ speed for 40 s, centrifuged for 10 min at 14,000×g. After centrifugation, the supernatant was transferred to 250 µL [protein precipitation solution (PPS)] in a 2.0 mL catch tube which was centrifuged for 5 min at 14,000×g to precipitate the protein. The supernatant was transferred to a 15 ml clean test tube with 1 ml binding matrix solution and rotated for 2 min, and then precipitated for 3 min to obtain the silica gel matrix. After removing

the supernatant and adding SEWS-M and air-drying, DNA was extracted and stored in a refrigerator at -20 °C.

PCR amplification

The extracted DNA was separated electrophoretically in 1% agarose to detect whether the extracted DNA was in accordance with the sequencing requirements. Before amplification of the target genes, a random bacterial and fungal DNA sample was selected to test whether the target gene was amplified and with a minimum number of cycles.

For bacteria, the 16S V3-V4 region was amplified using primer pair 338F (ACTCCTACGGGAGGCAGCAG)/806R (GGACTACHVGGGTWTCTAAT), Trans-Start Fastpfu DNA polymerase (Beijing TransGen Biological Co., Beijing) with 30 ng DNA sample, 1 µL forward primer (5 µM), 1 µL reverse primer (5 µM), 3 µL BSA (2 ng µL⁻¹), 12.5 µL 2×Taq PCR MasterMix and 7.5 µL ddH₂O. Cycling conditions were 95 °C for 5 min; 28 cycles of 95 °C for 45 s, 55 °C for 45 s and extended at 72 °C for 45 s; 10 min at 72 °C. PCR amplification products (target genes) were preserved at 4 °C and sequenced as soon as possible.

For fungi, the ITS1-ITS2 region was amplified using primer pair ITS1-F (CTTGGTCAATTAGAGGAAGTAA)/ITS2 (TGCGTTCTTCATCGATGC) and the same polymerase and PCR system used for bacteria, except 32 cycles were used.

DNA high-throughput sequencing

After electrophoretic detection and quality control, the amplified fragments were used to construct a Miseq library using the NEBNext Ultr DNA Library Prep Kit for Illumina and the standard protocol. The amplified fragment and the expanded sublibrary were then sequenced using the Illumina MiSeq PE300 platform (Illumina, San Diego, CA, USA). The PE reads obtained were spliced according to the overlap relationship between PE reads, and the sequence quality was controlled and filtered. According to the barcode and primer information at both ends of the sequence, the effective sequence was distinguished, the sequence direction was corrected, and the optimized sequence was obtained by using software FLASH and Trimmomatic. These steps were carried out by the Allwegene Technology Company in Beijing.

Statistical analyses

We used Pearson correlation analysis to assess correlations between the severity of decay and each of the soil property indicators, and a one-sample *t*-test to analyze differences between soil fungal diversity in the root zone of decaying and healthy sample trees. Multiple linear regression was used to construct the optimum regression equation relating the severity of tree decay with soil microbial quantity indicators, which were the dependent and independent variables, respectively. Path analysis was employed to compare the effect of different kinds of soil microorganisms on tree decay. All statistical analyses were done using SPSS 22.0 (IBM Co., Armonk, NY, USA).

Results

Tree trunk decay severity

Decay changes wood properties and decreases its quality. Mass loss in the wood core is usually used as a true value of wood decay (Liu et al. 2015). The mass loss data for 10 sample trees is considered as decay severity (Table 2). The mass loss of the wood core C2 was 0 i.e., from a healthy tree. Cores R3 and S2 had the most severe decay with mass loss of 47.1% and 48.3%, respectively.

Soil chemical properties versus trunk decay severity of standing trees

As the degree of decay increased for the sample trees, total N and K in the root zone soil rhizosphere gradually increased (Fig. 1a). Each was higher than the total P which changed slightly as decay severity increased. Similarly, hydrolytic N and available K were higher than available P in the root zone soil and showed a slightly increasing trend (Fig. 1b). Thus, these elements were more abundant in the root soil around decaying standing trees than around healthy trees, and might be due to their release from decaying litter and their incorporation into soil organic matter. In addition, there was more decaying litter under decaying trees. Reduced uptake of nutrients from the soil might also contribute to the increase in these elements.

Fig. 1 Relation between tree decay severity and soil chemical properties

Table 2 Decay severity as percentage loss in mass in 10 wood cores from sample trees

Variable	A2	B2	C2	D3	E3	F3	G1	H3	R3	S2
Decay severity (%)	14.56	18.35	0	9.18	29.57	39.90	34.84	25.76	47.11	48.30

Each core is coded with a letter for the tree and the core number; one healthy (C2) and nine decaying trees were analyzed

Soil microbial quantity versus trunk decay severity of standing trees

The distribution of soil microorganisms around the roots of sample trees with different decay severities was: bacteria > actinomycetes > fungi, with the number of bacteria (BN) $10-10^4$ times higher than actinomycetes, and 10^3-10^5 times higher than fungi (Fig. 2). Thus, bacteria were clearly the key contributors to soil microbial activity. As the severity of decay increased, the number of microbes of all microbial types increased gradually (Fig. 2), indicating decay severity might be closely related to the community of root soil microorganisms.

Multiple linear regression analysis was used to analyze the relationship between decay severity and root soil microbial abundance. The regression result is $y = -18.457 + 3.564 \times 10^{-4} \times 2 + 7.683 \times 10^{-5} \times 3$ ($R^2 = 0.664$, $p = 0.000$), where y is tree decay severity (Wood core WLR), $\times 2$ is the number of fungi (FN) and $\times 3$ is the number of actinomycetes (AN). The equation shows that decay severity was mainly related to the number of FN and AN in the soil.

Fig. 2 Relation between tree decay severity and soil microbial quantity

Fig. 3 Rarefaction measure PD_whole_tree curves for **a** fungi and **b** bacteria

However, a low determination coefficient ($R^2 = 0.664$) and a large residual factor [$e = 0.5(1 - R) = 0.336$] were also found, which indicates that other factors might exist in addition to FN and AN.

In the path analysis to further study the effect of soil microorganisms on tree decay, the direct path coefficient (DPC, P_{iy}) between any independent variable x_i and dependent variable y is the standard coefficient of the linear regression equation, and the indirect path coefficient (IPC, P_{xi-xj}) is the product of the Pearson correlation coefficient (r_{ij}) and DPC (P_{jy}) of any independent variable x_i to y . The DPCs of BN, FN and AN on decay severity was -0.305 , 0.397 and 0.413 , respectively, indicating that the direct effect of bacteria on decay severity was negative and not significant ($p = 0.113$), but the direct effects of fungi and actinomycetes on decay severity were positive.

Based on the Pearson correlation coefficient, the IPCs between soil microbe numbers and decay severity were calculated. The greatest negative IPC, -0.209 , was found between BN and FN, indicating that BN could affect FN in the soil and then might indirectly inhibit tree decay. All other IPCs between soil microbes were positive; the greatest positive IPC, 0.211 , was between AN and FN, which meant that soil AN could accelerate tree decay by affecting FN. The IPC between AN and BN was the smallest (0.117).

Soil microbial diversity versus trunk decay severity of standing trees

Soil microbial species diversity

α -Diversity represents the richness and evenness of different species of microorganisms. PD_whole_tree curve and Rank_abundance curve were applied to characterize the α -diversity of species (Figs. 3, 4).

PD_whole_tree is the sum of the branching lengths in the phylogenetic trees constructed using all groups in

the sample (Goedert et al. 2015). The higher the PD_{whole_tree} value, the greater the species richness in the sample. For both fungal and bacteria species, F3 was the most abundant (fungi: PD_{whole_tree} value > 175; bacteria: > 80) and D3 was the lowest in species number and diversity.

For the Rank_abundance curve to analyze species diversity, the shape reflects the homogeneity of species composition. The slower the downward trend of the curve, the higher the uniformity of the species composition (Bates et al. 2013). For fungi and bacteria, the curve decreased rapidly and steeply, indicating that the dominant flora in the samples accounted for a higher proportion and the species distribution was not uniform (Fig. 4).

Statistical analyses for relation between soil microbial diversity and tree decay

α -Diversity represents the richness of organisms in a particular region or ecosystem. The commonly used indices are the Chao1 index, PD_{whole_tree} index and Shannon index. In the Pearson correlation analysis of the relationship between decay severity of *P. koraiensis* and soil microbial α -diversity, there was no significant correlation between fungal α -diversity and decay severity. The same results were found for bacterial α -diversity (Table 3).

One-sample *t*-test was employed to analyze the relation between fungal PD_{whole_tree} index and bacterial PD_{whole_tree} index; the results show that there was no significant difference between decay and healthy tree with a value in fungal diversity (2-tailed, $p=0.062$) and in bacterial diversity (2-tailed, $p=0.195$), shown in Table 4.

Fig. 4 Rank_abundance curves of fungi and bacteria

Table 3 Pearson correlation analysis of microbial diversity and decay severity of sample trees

Microbial group	Chao1		Shannon		PD _{whole_tree}	
	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>
Fungi	− 0.149	0.682	0.285	0.425	− 0.279	0.435
Bacteria	0.019	0.958	− 0.250	0.486	0.010	0.977

PD is phylogenetic diversity. PD_{whole_tree} is the sum of the branching lengths in the phylogenetic trees constructed using all groups in the sample

Table 4 One-sample *t*-test of PD_{whole_tree} for bacteria

Microbe group	<i>t</i>	df	<i>p</i> (2-tailed)	Mean difference	SE	95% confidence interval	
						Lower	Upper
Fungi	157.269	8	0.062	− 33.176	− 2.173	− 68.390	2.038
Bacteria	78.732	8	0.195	− 3.838	− 1.413	− 10.102	2.426

PD is phylogenetic diversity; PD_{whole_tree} the sum of the branching lengths in the phylogenetic trees constructed using all groups in the sample; SE is standard error

Fig. 5 The unifracs_distance graph for species in various fungal phyla, Cercozoa, and others

Identification and analysis of dominant microbial species

Species richness, the number of individuals of each species in the samples, is obtained from the operational taxonomic units (OTUs), while the abundance of OTUs can be calculated from the number of sequences in OTU. Through high-throughput sequencing, seven known phyla and 203 known species were found in soil samples from the rhizosphere around the roots. Among all species, 21 had a relative abundance > 1%. The top three fungal phyla were Zygomycota (6.3%), Ascomycota (35.4%) and Basidiomycota

(55.4%), accounting for 97.1% of all fungi (Fig. 5). The relative abundance of agaricomycetes (Basidiomycota), the primary group associated with wood decay (Shang 2008), was 46.6%, but further statistical analysis showed that their abundance was not correlated with decay severity of standing trees ($R = -0.229$, $p = 0.525$). For other species, some are known soil inhabitants (*Cryptococcus podzolicus*; Liu 2016), others ectomycorrhizal fungi (*Inocybe maculata*; Larsson et al. 2009), and other pathogenic fungi (*Nectria ramulariae*). But their distribution in 10 samples was not correlated with decay severity.

Table 5 Fungal species with > 1% abundance and their relative abundance in 10 core samples (C2 is from a healthy tree)

Species	C2	D3	A2	B2	H3	E3	G1	F3	R3	S2
<i>Amanita argentea</i>	28.5066	0.0467	0.0643	0.0712	0.0000	0.0000	0.0000	0.0000	0.0026	0.0031
<i>Tricholoma terreum</i>	4.5484	0.0146	0.0253	0.0356	0.1339	0.0027	0.0000	0.0853	24.0095	0.1411
<i>Russula aff. integra_r_03014</i>	3.8724	0.0263	0.0298	0.0229	0.0000	0.0000	0.0000	0.0000	0.0026	0.0000
<i>Nectria ramulariae</i>	3.7250	0.0963	2.8694	1.8280	3.9643	1.1080	0.7216	9.7524	2.4210	0.5017
<i>Cryptococcus podzolicus</i>	2.8811	1.0683	4.8826	0.6686	2.2089	2.8020	2.4939	2.3609	1.1855	0.9125
<i>Tylospora fibrillosa</i>	0.0363	11.9968	0.0321	0.0966	0.0047	0.0054	0.0000	0.0000	0.0000	0.0031
<i>Russula aff. turci_r_04101</i>	0.0295	5.5226	0.0207	0.0254	0.0000	8.9306	0.0933	0.0000	0.0026	0.0000
<i>Piloderma byssinum</i>	0.0295	3.4765	0.0161	0.0381	0.0000	0.0000	0.0000	0.0000	0.0026	0.0000
<i>Amphinema sp_6_UK_2011</i>	0.0023	1.1997	0.0023	0.0051	0.0000	0.0000	0.0000	0.0000	0.0000	0.0031
<i>Cryptococcus terricola</i>	0.3630	1.0129	1.4209	0.0458	0.3924	0.7654	1.3991	0.5645	0.3030	1.3233
<i>Mortierella humilis</i>	0.3766	0.4145	2.5572	0.4119	1.2971	4.5549	1.3157	2.9154	1.1960	1.4613
<i>Mortierella camargensis</i>	0.2858	0.1255	2.6467	0.4500	1.3230	1.7155	0.2626	2.0523	2.5843	0.1662
<i>Russula viscida</i>	0.1066	0.1080	12.0309	0.1297	0.0047	0.0000	0.0000	0.0000	0.0026	0.0000
<i>Inocybe maculata</i>	0.1089	0.0671	0.0803	32.0316	0.0047	0.0000	0.0000	0.0075	0.0053	0.0031
<i>Inocybe glabrodisca</i>	0.0408	0.0292	0.0803	20.7917	0.0070	0.0000	0.0000	0.0000	0.0000	0.0063
<i>Inocybe rimosoides</i>	0.3108	0.0029	0.0000	0.0025	6.5680	0.0000	0.0000	0.0301	0.0342	0.0125
<i>Humicola nigrescens</i>	0.0181	0.0175	0.8080	0.0559	0.0775	3.4550	0.6063	0.6323	0.5796	0.1098
<i>Tomentella stuposa</i>	0.0045	0.5517	0.0069	0.0025	0.0094	0.0107	2.4153	0.0201	0.0000	0.0125
<i>Lactarius flexuosus</i>	0.0000	0.0029	0.0000	0.0000	0.0235	0.0000	0.0000	3.5678	0.0184	0.0094
<i>Thelephora palmata</i>	0.0000	0.0000	0.0390	0.0000	0.0047	0.0000	0.0000	0.0050	1.7940	0.0063
<i>Oidiodendron chlamydosporicum</i>	0.2064	0.0701	0.3581	0.0127	0.3830	0.8136	0.1325	0.2534	0.6349	1.4738

The relationship between relative abundance of the 203 species and the decay severity of *P. koraiensis* was further analyzed. Of all species, the number with more than 1% of relative abundance was 21, including *Amanita_argentea*, *Tricholoma_terreum*, *Russula_viscida*, *Russula_aff_integra_r_03014*. Their relative abundance in 10 samples was shown in Table 5.

In similar analyses for bacteria, species with relative abundance $\geq 1\%$ included Nitrospirae (1.78%), Bacteroidetes (4.32%), Gemmatimonadetes (4.34%), Chloroflexi (5.16%), Verrucomicrobia (7.36%), Actinobacteria (10.82%), Acidobacteria (30.03%) and Proteobacteria (33.34%), accounting for 97.15% of all bacteria. The relative abundance of different bacterial species was not correlated with various decay severities.

Conclusions

Higher levels of N, P, and K, especially total N and K, were found in the rhizosphere from decaying standing trees than from healthy standing trees.

As tree decay severity increased, the quantity of microorganisms in the soil around the tree roots increased gradually. Statistical analysis showed that decay severity was related to the number of fungi and actinomycetes. Soil bacteria may indirectly inhibit tree decay by affecting the number of fungi in the soil.

High-throughput sequencing and statistical analysis showed that decay severity of *Pinus koraiensis* was not correlated with soil microbial (fungal and bacterial) diversity.

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